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ABSTRACT

The intermittent intravenous administration of antibiotics by the syringe-infusion pump system is currently being promoted. To use this system, a dose of medication is prepared in 10-20ml of sterile water for injection and stored in plastic disposable syringes. At the time of administration, the syringe is placed in a syringe pump which infuses the solution via a micropore tube through a patient's primary intravenous line or heparin lock.

In order to use this system efficiently, the stability of the drugs must be known. Most stability information is based on the minibag or minibottle system, where the medication is diluted in 50-100ml of normal saline or 5% dextrose in water. Stability data on high concentrations (1-2gm/10-20ml) in sterile water for injection is very limited.

Five beta-lactam antibiotics (ampicillin, cefazolin, cefoxitin, piperacillin and ticarcillin) were studied at concentrations of 1-2gm/10-20ml of sterile water for injection and stored in plastic syringes at 24°C, 4°C and -15°C. The concentration of the antibiotic at several time intervals was determined by ultraviolet spectrophotometry and high-pressure liquid chromatography.

The degradation rate constants were determined and the time to degrade by 10% was calculated. The USP requires that a drug product contain at least 90% of the labeled amount. Thus, the time to degrade by 10% is used as the expiration time for these solutions. In addition, the Arrhenius equations for each drug were determined.

HPLC analysis showed that degradation products interferred with the UV spectrophotometric analysis of piperacillin, ticarcillin and ampicillin. Thus, the UV data could not be used. HPLC analysis of frozen solutions of piperacillin and ticarcillin showed no change at 3 months, while there was 93% loss of ampicillin at 3 months. HPLC showed that ticarcillin solutions lose 10% concentration at room temperature in 4 days and under refrigeration in 6 days. Piperacillin solutions lose 10% concentration at room temperature in 2 days and under refrigeration in 10 days.

UV analysis of cefazolin and cefoxitin was used since HPLC showed that degradation products do not interfere with UV spectrophotometry. Cefazolin solutions lose 10% of the initial concentration at room temperature in 13 days and under refrigeration in 30 days. Cefoxitin solutions lose 10% of its initial concentration in 2 days and under refrigeration in 23 days. Both frozen solutions retained 100% concentration at 3 months.

This study provides the pharmacist with information on the stability of these five antibiotics in sterile water for injection at the concentrations used in the syringe-infusion pump system. The pharmacist can use this system and ensure that patients are receiving the concentration of drug as required by USP.

STABILITY OF FIVE BETA-LACTAM ANTIBIOTICS
IN STERILE WATER FOR INJECTION AND STORED
IN PLASTIC SYRINGES

Thesis presented

by

Diane L. Borst

to

The Graduate School of Pharmacy and Allied Health Professions
in Partial Fulfillment of the Requirements for the Degree of

Doctor of Pharmacy

NORTHEASTERN UNIVERSITY
BOSTON, MASSACHUSETTS

June, 1984

NORTHEASTERN UNIVERSITY

GRADUATE SCHOOL OF PHARMACY AND ALLIED HEALTH PROFESSIONS

Thesis Title: Stability of five beta-lactam antibiotics in sterile water for injection and stored in plastic syringes

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Program: Doctor of Pharmacy

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The Lord God, the Creator of this world, who gives purpose to living and hope for better things to come.   Psalm 145, Colossians 1.

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ABSTRACT

The intermittent intravenous administration of antibiotics by the syringe-infusion pump system is currently being promoted. To use this system, a dose of medication is prepared in 10-20ml of sterile water for injection and stored in plastic disposable syringes. At the time of administration, the syringe is placed in a syringe pump which infuses the solution via a micropore tube through a patient's primary intravenous line or heparin lock.

In order to use this system efficiently, the stability of the drugs must be known. Most stability information is based on the minibag or minibottle system, where the medication is diluted in 50-100ml of normal saline or 5% dextrose in water. Stability data on high concentrations (1-2gm/10-20ml) in sterile water for injection is very limited.

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The degradation rate constants were determined and the time to degrade by 10% was calculated. The USP requires that a drug product contain at least 90% of the labeled amount. Thus, the time to degrade by 10% is used as the expiration time for these solutions. In addition, the Arrhenius equations for each drug were determined.

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This study provides the pharmacist with information on the stability of these five antibiotics in sterile water for injection at the concentrations used in the syringe-infusion pump system. The pharmacist can use this system and ensure that patients are receiving the concentration of drug as required by USP.

STATEMENT OF THE PROBLEM

Antibiotics are commonly administered by intermittent infusion. Direct intravenous injection, in-line volume control devices and piggyback systems with bags or bottles have been used. Recently, the intermittent intravenous administration of antibiotics via a syringe infusion pump system has been promoted. Antibiotics are administered in 10-20ml of sterile water for injection from a plastic syringe instead of in 50-100ml of normal saline or 5% dextrose in water from a plastic bag or glass bottle. The advantage of this system is primarily cost. Syringes and sterile water for injection are less expensive than minibags or bottles of dextrose or saline. Another major advantage is the constant infusion rate provided by the use of a pump.

With the use of antibiotics in syringes, one question that continually arises is whether or not the medication is stable. Many antibiotics have limited stability in solution and are marketed as powders for reconstitution. These medications are often prepared in the hospital pharmacy and delivered to patient care areas for administration. The time delay between preparation and administration must be short enough to ensure that the antibiotic has not degraded in solution to inactive and/or toxic products.

The information available on the stability of many antibiotics at high concentrations and in syringes is limited. Usually, reconstituted antibiotics are administered in 50-100ml of normal saline or dextrose. Most stability data available is based on this system. There is limited data on 5-10ml dilutions with sterile water for injection.

The purpose of this study is to determine the stability of five beta-lactam antibiotics (ampicillin sodium, cefazolin sodium, cefoxitin sodium, piperacillin sodium and ticarcillin disodium) when diluted with sterile water for injection (10-20ml) and stored in plastic disposable syringes at room temperature (24°C), under refrigeration (4°C) and when frozen (-15°C).

HYPOTHESIS

There is no change in the concentration of ampicillin, cefazolin, cefoxitin, piperacillin and ticarcillin when diluted with 10-20ml of sterile water for injection and stored in plastic disposable syringes at -15°C for 3 months and at 4°C and 24°C for 1 month.

REVIEW OF THE LITERATURE

Antibiotics are among the most commonly used medications in the acute care hospital. They also make up a high percentage of the pharmacy budget. The choice of an antibiotic, the route of administration and the correct dose are crucial in deciding how to treat a patient appropriately. Since the discovery of penicillin in the 1920s and the cephalosporins in the 1940s, chemical modification of the side chain of the beta-lactam ring has resulted in a multitude of beta-lactam antibiotics[1]. These antibiotics are among the most popular due to their low incidence of side effects and their clinical effectiveness against a wide variety of bacteria.

The beta-lactam ring structure is essential to microbiologic activity[1]. The different spectra of activity among this group is due to variations in the side chains. The beta-lactam ring, however, is unstable when exposed to water. Hydrolysis of the ring occurs resulting in the loss of activity. The rate of hydrolysis increases rapidly in the presence of hydrogen ions. Oral administration of some of these antibiotics is not possible due to the rapid hydrolysis in the acidic gastric fluids. Therefore, these antibiotics must be given parenterally.

INTRAVENOUS ADMINISTRATION

In the past, intravenous medications were given by direct intravenous injection by physicians[2]. As the number of medications increased and new technology developed, different methods of administering intravenous medications were attempted. One method was to administer the drugs by slow intravenous infusion in a larger volume of fluid[3,4]. Continuous infusions of antibiotics are possible but there are some disadvantages. Continuous infusions result in low antibiotic serum levels which may be less effective than higher levels achieved

through intermittent intravenous administration. Solutions were prepared with large volumes of fluid and infused over long periods of time. Loss of drug activity could occur during this prolonged infusion period. In addition, the risk of phlebitis may be increased with the constant infusion of drugs such as penicillin. Pencillin is very irritating to the veins and can result in inflamed, red, hot, tender tissues. This could lead to serious complications such as infection and necrosis.

The intermittent infusion of antibiotics was proposed as a better method due to higher blood levels that could be achieved. Direct intravenous push was one method but blood levels were often too high resulting in a higher incidence of side effects. For example, ampicillin should be given over a minimum of 10-15 minutes to avoid convulsions and muscle irritability[2,5,6]. In-line volume control sets were developed, such as the Soluset, Vol-u-trol, Buretrol and Metriset[2,3,7]. These intravenous sets contained in-line plastic containers capable of holding at least 100ml of fluid. The container would be filled with fluid which could then be infused over a short period of time. If medication was to be given, it could be mixed in the container and then administered. One disadvantage of this system was an increased risk of bacterial contamination of the fluids due to multiple injections into the plastic container[3]. Also, there was an increased risk of calculating and mixing an incorrect dose at the patient's bedside.

Another method uses a piggyback system[3,7]. A single dose of medication is prepared in a small glass bottle or plastic bag. When the antibiotic is to be administered, an intravenous line (the secondary set) is attached to the bottle or bag and then administered through the primary line.

Several factors such as cost, stability, compatibility, toxicity, contamination, ease of preparation, ease of administration and desired blood levels must

be considered when choosing among these methods of administration. Many studies have been published comparing these different methods[4,7-9]. The move to a pharmacy intravenous admixture service was begun in order to increase the quality control over these medications. Guidelines and recommendations for the proper handling of intravenous medications have been published[2,5,10-12]. These changes did not eliminate all the problems. Drug stability, cost-effectiveness and increased pharmacy workload were still problems to be solved.

The search for an accurate, simple, safe, cost-effective method is still underway[13]. In the past several years, the syringe-infusion pump system (3M Medifuse, Razel and Bard) has been tried[13,14]. This system consists of a pre-pared dose of an antibiotic in a syringe instead of a bag or bottle. The volume is relatively small (10-20ml) and the fluid used is sterile water for injection. In order to administer this medication, the syringe is placed in a special pump which is set to infuse the fluid over a specified period of time. It must be infused through a micropore line (because the volume of fluid is small) and then through the main intravenous line, similar to the piggyback system.

The pump is a good system because the costs of preparation are decreased[14], the ease of administration is increased[13] and continuous flow rates can be achieved independent of gravity and more expensive pumps and con-trollers. The questions concerning drug stability, contamination rate (bacterial and physical), phlebitis and ease of preparation must still be considered.

DRUG STABILITY

Knowledge of drug stability is important to ensure that the correct dose is administered. If the antibiotic is not administered immediately after preparation, the drug, particularly the beta-lactams, can degrade, resulting in an ineffective

dose or toxicity. The FDA requires detailed documentation from the manu-
facturer on the stability of their products in different packages, at different
temperatures and in different formulations. The USP states that for beta-lactam
antibiotics, the package must contain between 90%-115% of the labeled con-
tents[15-19]. The weakest link in ensuring the contents of these antibiotics
occurs after preparation for administration[20]. The responsibility for stability
after repackaging the product lies with the pharmacist[20].

The information on stability provided to the practicing pharmacist is
limited. Legally, accurate stability data should be available for each change in
the variables that affect stability[20]. There has been a tremendous quantity of
stability information published in recent years. Most stability studies done on
these antibiotics are at lower concentrations, in dextrose or saline, and in glass or
polyvinyl chloride (PVC) bags. In spite of these numerous reports, much infor-
mation is still needed because stability of a particular drug is dependent on so
many factors. In the case of the syringe-infusion pump system, the solution,
concentration and container have been changed. Manufacturers, such as Beecham
Labs, have not studied stability in syringes due to the wide variety of plastics and
rubbers used to make disposable syringes[21].

The degradation of a drug can be defined as an irreversible chemical change
in the structure of the organic molecule[20,22-24]. The most common reactions
are hydrolysis and oxidation of the drug molecule. Stability of a drug refers to
the rate at which these reactions proceed. Some beta-lactam antibiotics
hydrolyze faster than others and so are less stable. When these antibiotics are
prepared for intravenous administration, degradation can occur before the drug is
administered. These medications can be used only if they have not degraded by
more than 10% of their labeled concentration as required by the USP.

KINETIC PRINCIPLES OF STABILITY

The analysis of drug stability is possible through the application of the principles of chemical kinetics, such as reaction rates and kinetic equations[20,22,23]. The variables that affect these rates include concentration, temperature, pH, catalysts and radiation energy[20,23,24].

The relationship between the concentration of the drug and the reaction rate is expressed as the order of the reaction. Zero indicates that the reaction rate is independent of the concentration of the drug. First order reactions are those in which the rate is dependent on the concentration of one reactant, i.e., the drug molecule. Pseudo-first order refers to the situation where ordinarily the reaction rate is dependent on the concentration of the two reactants, but one is in such excess that the rate appears to be dependent on the concentration of the other reactant. Thus, it appears to be first order. Some drugs have very complicated degradation mechanisms but usually zero, first or psuedo-first order can characterize the reaction rate[20].

Knowledge of the order of the reaction enables one to determine the rate of degradation of the drug. The information needed[23] is the initial concentration of the drug and then the concentration of the drug at two or more time intervals. For a zero order reaction, the graph of these points (concentration-time) should yield a straight line. The equation for this zero order reaction is:

$$C = C_0 - kt.$$

A first order or pseudo-first order process is linear on semi-log paper and the equation is:

$$\log C = \log C_0 - \frac{kt}{2.303}.$$

Once k, the rate constant, and C_0, the initial concentration, are known, then the

concentration, C, can be determined at any time, t. To find the time for 10% degradation[23], the equation for a first order process is:

$$t = \frac{.104}{k}$$

and this would establish the expiration date for that drug based on USP requirements.

The temperature at which the antibiotics are kept affects the rate of degradation[20,22,23]. The Arrhenius equation,

$$k = Ae^{\frac{(-Ha)}{RT}},$$

describes this effect. (k, rate constant; A, proportionality constant; Ha, heat of activation; R, the gas constant; and T, temperature in degrees Kelvin.) The relationship between temperature and the rate constant can be determined by plotting k at different temperatures against $\frac{1}{T}$. The equation resulting from the straight line is:

$$\log k = \log A - \frac{Ha}{2.303 RT} \cdot \frac{1}{T}.$$

This equation can then be used to determine the rate constant at any temperature. Generally, an increase in temperature will increase the rate of degradation and a decrease in temperature will decrease the rate of degradation.

Many reactions are affected by pH. The mechanism and rate of degradation can depend on the presence of hydronium and hydroxyl ions. Ampicillin has a much slower degradation rate at an acid pH (5-7) than at an alkaline pH (7-9)[25]. pH - Rate profiles have been prepared for many drugs. Most of the beta-lactams have the slowest rate of degradation at a neutral pH.[23,24]

The solvent used can also affect the rate of the reaction. Hydrolysis is the major cause of instability and occurs by nucleophilic attack of water on the drug molecule causing a split in the molecule. The most common functional groups

involved are lactams, esters, amides and imines. This mechanism usually follows first order kinetics[24]. Penicillins, in dextrose solutions, form pen.cilloyl-carbohydrate conjugates[26] and degrade faster than in saline solutions[27].

Oxidation of a drug molecule is the second most common cause of degradation[20]. Autoxidation occurs spontaneously with the addition of atmospheric oxygen. Both oxidation and hydrolysis can be catalyzed by light, particularly ultra-violet radiation. The beta-lactams generally do not undergo oxidation nor are they affected by light.

PLASTICS AND INTRAVENOUS MEDICATIONS

The container that a medication is stored in does not necessarily affect the stability of the drug molecule but it can alter the concentration of the solution and the purity of the solution[20,24,28-31]. Plastics have been widely used in medicine and pharmacy. Polyvinyl chloride is used in making intravenous tubing, catheters and solution containers. Problems associated with the use of plastics have been known since the 1960s[31,32]. Autian described five problems associated with plastic containers such as leaching, sorption, permeation, chemical reactivity and changes in physical properties of the plastic[32].

Plastics are not necessarily harmful but there are many additives used to prepare the final product[31,32]. Stabilizers have been used to prevent oxidation and saturation of the double bonds of the polymer. Plasticizers have been used to make the final PVC product soft and pliable. Rubber, also used in plastic containers and syringes, contains chemicals such as curing agents, reinforcing agents, accelerators, pigments, antigradients and substances used to vulcanize the rubber, to increase elasticity and durability[33].

Leaching of materials from plastics or rubber to the solutions is a major concern. It has been reported to occur with the storage of blood products in PVC bags, during hemodialysis, and storage of intravenous fluids in bags[31,33-36]. Di-2-ethylhexyl phthalate (DEHP), a plasticizer, has been found in human blood stored in plastic PVC bags[34,35]. Studies using plastic bags with normal saline, dextrose, or sterile water for injection have shown minimal leaching of DEHP. Fluids that are lipophilic, have lipoproteins or blood have much more leaching[36,37]. Symptoms and toxic effects in humans from the use of these containers have not been identified yet, but may be cause for concern.

Di-n-butyl phthalate (DBP) is another chemical that has been found in small quantities in solutions of saline or dextrose but not in blood products stored in bags[35]. The clinical significance of this chemical is also unknown.

Leaching of materials from rubber has also been identified. Benzothiazole is one chemical that can leach out from the rubber plunger-seal of some plastic syringes[38]. Fourteen other chemicals have been identified that leached into intravenous solutions from two different rubber stoppers of large volume parenterals[33]. Quantitative analysis was not done.

Kowaluk et al.[39] studied the problem of leaching of chemicals from plastics, including disposable plastic syringes. They used two different brands of plastic syringes. Solutions stored in one brand (Top brand), which has a rubber plunger seal, was found to have a contaminant, 2-(2-hydroxyethylmercapto)benzo-thiazole. This compound is the possible product of a reaction between 2-mercaptobenzothiazole, a rubber processing chemical, and ethylene oxide, used to sterilize the syringes. The other syringe that was studied, Pharma-Plast, did not have rubber parts and did not cause any problem with leaching.

Sorption is the second major problem associated with the use of plastics. Adsorption refers to the binding of the drug molecules to the plastic surface while absorption refers to the penetration of the drug molecule into the plastic matrix itself. Nitroglycerin, diazepam, insulin, heparin, vitamin A, thiopental and warfarin sodium solutions all lose potency when stored in PVC containers due to sorption[28,39-41]. The extent of ionization and degree of lipophilicity of the molecule determines the extent of sorption to plastic bags. The more lipophilic, the greater the degree of sorption[40].

Kowaluk et al.[39] studied the interaction between drugs and plastic intravenous delivery systems. Of the 45 drugs studied, they found that there was no loss of drug after storage in plastic disposable syringes after 24 hours at room temperature. Ampicillin and cefoxitin were among the drugs studied.

Permeation, chemical reactivity and altered physical properties of plastic are less common problems. Permeation refers to the passage of gas or vapors through the plastic from the solution to the environment or from the environment into the solution. Chemical reactivity is the term Autian used in those few instances where the solute or the solvent caused the plastic to deteriorate. The alteration in physical properties of the plastic, such as tensile strength and pliability, could also occur.

ADVERSE EFFECTS OF BETA-LACTAM DEGRADATION PRODUCTS

The knowledge of the stability of an antibiotic in solution is not only important in assuring that the desired dose is given to a patient, but it is also important in preventing adverse effects. Most medications degrade to inactive compounds. Most of the penicillin and cephalosporin degradation products are inactive as antibacterial agents but some have been shown to be the cause of

allergic reactions[26,42,43]. Four factors[26,44-47] have been proposed to be the cause of these allergic reactions to penicillins: 1) intact molecule, 2) degradation products, 3) impurities and 4) metabolites.

The degradation products such as penicillenic acid and penicillin polymers have been shown to cause wheal and flare reactions in sensitized animals and patients. Many penicillins begin to polymerize with degradation products while others, such as ampicillin, autopolymerize. Ampicillin polymerization increases with increasing concentration. The presence of tetramers (0.1%) of ampicillin in 20% solutions occurs within 30 minutes[26]. Polymers are immunogenic but the significance has not been established. Other degradation products have been implicated in this adverse reaction but the significance is not known[43].

Various proteins and other contaminants from the preparation of these anti-biotics may also be immunogenic[43]. The significance of these is questioned since the commercially available products have extremely low levels of these contaminants.

The various metabolites are similar to the degradation products of the peni-cillins. Penicillenic acid, also formed in vivo, has been suggested to be the main cause for this adverse reaction[42]. The true cause or causes for the allergic reactions is not presently known.

BETA-LACTAM STABILITY

The penicillins and cephalosporins are similar in that both have the beta-lactam ring in their structure (Figure I). This ring is very unstable in solution and hydrolysis occurs, resulting in loss of antibacterial activity[1,26]. The side chains

have been altered resulting in a change in antimicrobial activity and physical-chemical variables such as stability[1,24].

Pencillins

Pencillin G undergoes hydrolysis very rapidly in solution. Multiple degradation products and pathways have been proposed as shown in Figure II[43,48]. Different products are formed in acid and alkaline solutions. The beta-lactam ring is susceptible to attack by hydroxyl ions, resulting in the opening of the beta-lactam ring. Penicilloic acid is formed and with the loss of carbon dioxide, penilloic acid is formed. In the presence of hydrogen ion, the side chain is lost forming penillic acid. One of the major degradation products is penicillenic acid. In a higher concentration of hydrogen ion, penaldic acid and penicillamine are formed. The loss of carbon dioxide results in penilloaldehyde. The beta-lactam ring is also susceptible to attack by metal ions, penicillinases, organic catalysts and water[44,49].

Ampicillin

Ampicillin, alpha-aminobenzylpenicillin, is 200 times more stable than penicillin G in solution[25]. The amino side chain plays a role in the rate of degradation of the ampicillin molecule but not in the mechanism[25]. Hydrolysis of the beta-lactam ring is still responsible for the overall degradation[25]. Penicillin is degraded by intramolecular attack of the side chain on the beta-lactam ring. The electron withdrawing effect of the amide group of the ampicillin side chain is responsible for the decreased reaction rate of ampicillin[50].

The products of ampicillin degradation in solution are shown in Figure III. The rate and mechanism of degradation vary with the pH. The rate is slowest at a pH of 7.5[51]. Since ampicillin is an amphoteric molecule, the cation, anion, and

zwitterion are present in varying amounts depending on the pH of the solution. The concentrations of the degradation products will vary with the solution pH. In acidic solution, alpha-aminobenzylpenicillinic acid is formed. At a lower pH, alpha-aminobenzylpenamaldic acid is formed. In conrast, under basic conditions, alpha-aminobenzylpenilloic acid and alpha-aminobenzylpenicilloic acid are formed. Carbon dioxide is also released during this process.

Tsuji and Robertson[52] analyzed the degradation of ampicillin in basic solutions using an HPLC assay. After one week in alkaline solution, they identified alpha-aminobenzylpenicillenic acid and alpha-aminobenzylpenicilloic acid on the HPLC chromatogram. Other products such as penicillanic acid did not absorb ultraviolet light at 254nm and so did not show on the chromatogram.

Another stability problem with ampicillin is the formation of polymers after storage at room temperature for a few days[53,54]. Polymers of a maximum size of 8 molecules are due to the nucleophilic attack of the amino side chain onto the beta-lactam ring of another molecule. As mentioned before, polymers have been implicated as a cause for allergic reactions[26].

The solvent is a major factor in affecting the degradation of ampicillin, either by a change in pH or by providing a catalyst[55]. Dextrose solutions are a well known problem. When ampicillin is stored in dextrose, 10% loss can occur within 2 hours at room temperature and within 4 hours when refrigerated[6]. However, ampicillin in normal saline is stable for longer periods of time. At room temperature, solutions of ampicillin in either normal saline or water are stable for up to 8 hours[6]. When refrigerated, solutions are stable for up to 48 hours.

The concentration of ampicillin in solution is another important factor in the stability of this antibiotic[6,56]. Ampicillin degradation follows first order kinetics; it is less stable at higher concentrations. One manufacturer specifically states stability in terms of concentration[6]. This concentration effect is not as dramatic with the other beta-lactams.

Decreasing the storage temperature usually lowers the rate of degradation. An exception is frozen solutions of ampicillin. Holmes et al.[57] studied the stability of ampicillin 1gm in 50ml bags of normal saline (NS) or 5% dextrose in water (D5W). They used a microbiologic assay to determine the stability after freezing solutions at -20°C, -30°C and -70°C for 30 days. Ampicillin was stable in NS if stored at -30°C or -70°C but not at -20°C. Ampicillin in D5W was not stable at -20°C or -30°C but could be stored at -70°C. Unfortunately, freezing at -70°C is not practical in the typical pharmacy.

Dinel et al.[58] studied ampicillin in 50ml of D5W or NS bags frozen at -20°C. The samples were assayed after thawing at room temperature for 3 hours. When stored in NS, ampicillin was stable for up to one day. However, in D5W, ampicillin lost 50% of its initial concentration within one day. They concluded that frozen solutions of ampicillin were not stable.

There has been some variation in the results of stability studies over the years. Gallelli et al.[59] reported that 0.5% ampicillin in NS was stable with 100% activity after storage at room temperature for 14 days and at 5°C for 60 days. Warren et al.[60] reviewed this study and suggested that Gallelli's results differed from his because of two possible reasons. First, Gallelli used a twofold broth dilution bioassay which had been shown to be unable to detect ampicillin inacti-

vation of 50%. Second, Gallelli also used very low concentrations of ampicillin (0.5%) while the usual concentrations used are at least 2%.

The stability data from the literature for ampicillin in bags or bottles is summarized in Table I. Room temperature solutions must be used within one to eight hours. Refrigerated solutions must be used within 4 to 72 hours. The data for these studies varies due to the different concentrations, solvents and assays used.

The information available on the use of ampicillin in syringes and sterile water is limited. The concentration in the syringe infusion pump system is 1-2gm/10ml, almost ten times as concentrated as most solutions studied. The stability at these concentrations is unknown.

Piperacillin

Piperacillin is a new derivative of 6-aminopenicillanic acid with broad antibacterial coverage. There is limited information on the stability of piperacillin, available only from Lederle Labs. Piperacillin is stable in D5W and NS for 24 hours at room temperature, up to one week refrigerated and up to one month frozen in both glass and plastic containers[61]. Table II gives a more detailed listing of the company's data of piperacillin in PVC bags, glass bottles and plastic syringes at various temperatures, concentrations and solvents. Piperacillin solutions in plastic syringes have only been studied after freezing. A 40% solution is stable for up to 32 days[61,62].

Ticarcillin

Ticarcillin is also a semisynthetic derivative of 6-aminopenicillanic acid. The commercial preparation is a mixture of D- and L- isomers, readily identified

by HPLC[63]. The activity against gram negative organisms, particularly Pseudomonas aeruginosa, and its resistance to beta-lactamase is attributed to the carboxyl group of the alpha carbon of the side chain[64].

The degradation products of ticarcillin have not been fully identified but can be detected by ultraviolet spectrophotometry as shifts in the absorbance peaks[21]. The stability of ticarcillin in syringes has not been assessed by the company because of the differences in the syringes available[21]. In general, the stability of concentrations of 2-3gm/10-20ml in glass ranges from 12-72 hours at room temperature, 1-14 days when refrigerated and up to 30 days when frozen[21]. A summary of other stability data is shown in Table III.

Lynn[64] used a microbiologic assay to study the stability of 500mg/ml solutions of ticarcillin in water. Stability was maintained for 24 hours at room temperature and up to seven days at 5°C. A 2% solution in NS or D5W was also stable for up to 24 hours at room temperature. The containers were not specified.

Holmes et al.[57] also used a microbiologic assay to study the effect of freezing and microwave thawing on the stability of ticarcillin stored in PVC bags. They found that 3 grams in 50ml of D5W, stored at -20°C for 30 days, maintained 90% of its initial activity after room thawing and microwave thawing.

Gupta and Stewart[63] developed an HPLC assay to study the chemical stability of ticarcillin 2% in D5W or NS in PVC bags. They found that room temperature samples remained stable for 24 hours. The refrigerated samples remained stable for at least 15 days (93% potency left).

In contrast, the manufacturer[65] has recently stated that ticarcillin solutions are stable for up to 3 days at room temperature. The concentrations studied were up to 10-100mg/ml in NS, D5W and SWI. Refrigerated solutions are stable for up to 14 days. Higher concentrations (1gm/2ml, 3gm/6ml) should be used promptly. Frozen solutions (10-100mg/ml) are stable for up to 30 days. Information on the stability of ticarcillin in water, plastic syringes and high concentration is not available.

Cephalosporins

The cephalosporins are similar to penicillins except there is a six membered ring (dihydrothiazine) adjacent to the beta-lactam ring. The nucleus is 7-amino-cephalosporanic acid. The degradation of cephalosporins differs from the penicillins in that the hydrolysis products are rapidly degraded[49]. Degradation reactions follow first order kinetics and the beta-lactam ring opening can be followed by ultraviolet spectrophotometry at 260nm[66].

Cefazolin

Cefazolin is a first generation semisynthetic cephalosporin. Initial beta-lactam hydrolysis is responsible for overall degradation in aqueous solutions[67]. Stable compounds analogous to penicillin hydrolysis products do not remain but are rapidly broken down[66]. Cefazolin degradation varies with pH, the slowest rate occurs over a pH range of 5-7[66,67].

Some manufacturers (SKF[68], LILLY[69]) state that solutions in SWI, NS and D5W are stable for up to one day at room temperature and for up to four days when refrigerated, regardless of concentration and container. Frozen solutions in the original container are stable for up to 12 weeks[69].

Gupta and Stewart[63] studied the stability of cefazolin using an HPLC assay. Their purpose was to supplement the limited information from the manufacturers. Cefazolin, 2%, in PVC bags of NS or D5W was stable for up to 15 days in NS and D5W when refrigerated. This is much longer than the manufacturer's suggested times.

Bornstein et al.[70] studied cefazolin in different fluids using a microbiologic assay. Generally, they found that cefazolin was stable for up to one week at 5°C and 25°C. More specifically, when cefazolin was prepared in SWI at a concentration of 1gm/4ml, it was stable for up to 4 days at room temperature and 14 days when refrigerated. All samples were stored in glass containers. At a concentration of 0.5% in NS, it was stable for up to 7 days when refrigerated and at room temperature. When stored in D5W, it was stable for up to 14 days when refrigerated and 4 days at room temperature. They stated that the manufacturer's recommended expiration dates are shorter because of the concern for bacterial contamination and growth in the solutions.

Carone et al.[71] studied the stability of frozen cefazolin solutions. Concentrations of 1gm/2.5ml and 10gm/45ml were prepared with SWI, D5W and NS. They were stored in glass containers between -10°C and -20°C. These solutions were stable for up to 26 weeks, based on a microbiologic assay. Solutions in NS or SWI (0.5%) were stable for up to 12 weeks.

Dinel et al.[58] also studied frozen solutions of cefazolin in PVC bags of NS or D5W. A 0.5% solution was stable for up to 30 days when frozen and then for up to 21 hours after thawing if kept in the refrigerator.

Kleinberg et al.[72] also used a microbiologic assay to study cefazolin stability when frozen in Hy-Pod hypodermic syringes. They used a 1gm/3ml solution of SWI and stored at -20°C. Samples were stable for up to nine months. In their discussion, they warned that it cannot be assumed that this stability data can be used when cefazolin is stored in any other syringe or container.

Frozen solutions were usually thawed by being exposed to room temperature for several hours. Tomecko et al.[73] studied the effect of using microwave ovens to thaw the frozen solutions. Cefazolin was prepared as a 1% solution and frozen for 48 hours. The D5W and NS solutions were then thawed in the microwave oven for 50 to 220 seconds. The solutions were assayed microbiologically and found to retain 90% of their initial activity. There was no difference between microwave thawing and room temperature thawing.

Cefoxitin

Cefoxitin is a derivative of Cephamycin C, produced by Streptomyces lactamdurans[74]. It is active against a broad range of bacteria and is resistant to destruction by beta-lactamases. The methoxyl group on the 7-alpha carbon is responsible for this resistance[75]. It is chemically stable in dry form for 3 years if protected from moisture[74]. It is relatively unstable in solution due to hydrolysis of the beta-lactam ring[74,76]. The initial beta-lactam hydrolysis products are unstable[74]. Some of these secondary products have been identified.

The rate of degradation follows apparent first order kinetics over the pH range of 3-9[74]. The maximum stability is attained over the pH range of 5-7. In commonly used intravenous fluids, there is approximately a 10% loss of activity in 2 days at 25°C, 30 days at 5°C, and at least 30 weeks at 0°C[74,75]. A summary of the stability data is given in Table V.

The manufacturer states that all solutions are stable for 24 hours at room temperature[77]. Refrigerated solutions in the original container are stable for one week. They recommend an expiration date of 48 hours for any solution that is further diluted and stored in the refrigerator. Solutions of cefoxitin in sterile water for injection in plastic syringes was stated to be stable for up to 24 hours at room temperature, 48 hours if refrigerated and 30 weeks if frozen.

Gupta and Stewart[78] studied the stability of cefoxitin under conditions of the typical intravenous admixture service. Cefoxitin wa prepared as 2% solutions in NS or D5W, in PVC bags, and stored at 24°C and 4°C. They found no difference between the two solvents. The 24-hour expiration date for room temperature storage was reasonable, but they found that the refrigerated samples had 96% of the initial concentration on day 13 and 89% on day 44. This is six times longer than the 7 days recommended by the manufacturer.

O'Brien et al.[75] stated that cefoxitin was stable for up to 24 hours at room temperature and for up to 30 days if refrigerated, regardless of solution, concentration and container. Frozen samples, at a concentration of 90mg/ml, in D5W, NS or SWI and in the original container were stable for up to 30 weeks. Stability after thawing was studied at room temperature and after refrigeration. These solutions were stable for up to one week at 5°C and for up to 24 hours when left at room temperature. They also studied a 1gm/10ml solution in SWI stored in plastic syringes. At room temperature, it retained 94% of the initial concentration at 24 hours and 89% concentration at 48 hours.

Stiles[79] prepared 0.5% and 1% solutions in D5W and NS in PVC bags. He froze these at -20°C for 72 hours and thawed via microwave radiation. HPLC analysis of the solutions before and after showed no significant difference.

Cefoxitin was one of the 46 injectable drugs studied for interaction with PVC bags[40]. Cefoxitin was stored in PVC and glass up to one week at room temperature. Analysis by UV spectrophotometry revealed no difference between the concentration in PVC bags versus glass bottles.

METHODS OF ANALYSIS

The method of analysis in a stability study is crucial to determine the true rate of degradation. There are a multitude of methods to choose from, each with its own advantages and disadvantages. The method of choice is based on specificity, sensitivity, accuracy, cost and analysis time[80].

The method chosen must be specific for the drug studied. This is important when a sample with more than one drug must be studied. The method in a stability study must be able to distinguish the parent compound from the degradation products. If using absorbance spectrophotometry, the degradation products must not absorb at the same wavelength as the parent compound. The method of analysis must be stability-indicating[81].

The sensitivity of an assay refers to the lowest concentrations of drug that can be detected. Many assays can not be used to quantitate blood levels of a drug because the sample size is too small and the concentrations are too low. If the concentration of a drug is in ng/ml, the assay used must be able to detect the drug at this low concentration. This is not a big factor in stability studies where the concentration of drug is not very low.

The accuracy required varies with the goal of the study. One goal of a stability study is the detection of 10% loss of concentration of a drug. The micro-biologic assays used in some studies have error rates of $\pm$ 5-10%[72]. It is difficult to accurately determine the time at which there is 10% loss if the assay varies $\pm$ 10%. A method of analysis can also be too accurate. It is unnecessary to use a test that detects a 0.01% change if the only purpose is to detect a 10% change.

Ultraviolet Spectrophotometry

Direct ultraviolet (UV) spectrophotometry is a good assay method because of its speed, simplicity, sensitivity and low cost. Absorption spectrophotometry is defined as the measurement of an interaction between electromagnetic radiation and molecules or atoms of a chemical substance[82]. UV radiation (190-380nm) is most often used in drug analysis because of its greater accuracy and sensitivity when compared to infrared radiation. Solutions of 10ug/ml will absorb well in the UV region, whereas concentrations of 1-100mg/ml may be required for sufficient absorbance in the infrared range[82]. UV spectrophotometry is not as specific as other methods for quantitative analysis. This is a major disadvantage for stability studies when the parent compound and degradation products absorb at the same wavelength. However, UV spectrophotometry is used in USP recommended analysis for identification and content of drug products[83].

The use of UV spectrophotometry[84] in quantitative analysis is based on Beer's Law which states that absorbance of electromagnetic radiation by a solution is directly proportional to the concentration of the solution. (A = abc; where A is absorbance, a is the absorptivity constant, b is the distance radiation passes through the solution and c is the concentration.)

A UV spectrophotometer consists of an electromagnetic source, a sample holder, detector and recorder (Figure IV). Monochromatic light (light of one wavelength) is directed through a solution to a photoelectric element which measures the light transmitted (not absorbed). Transmittance or absorbance is then displayed on a digital display, a meter or recorded on paper.

The first step in using UV spectrophotometry for quantitative analysis of a compound is to pick a wavelength where the compound absorbs maximally. Using

this wavelength assures the operator of getting a good absorbance reading during analysis and will also decrease error. Since the rate of change of absorbance at the peak is minimal, the error is minimized if there is a slight change in wavelength used.

The next step is to develop a standard curve. Various solutions of known concentration are prepared and the UV absorbance is measured. This data is plotted (absorbance vs. concentration) and a straight line should result since absorbance and concentration are directly proportional (Beer's Law). This graph or the equation of the line is used to convert any absorbance read by the machine to the corresponding concentration of the solution.

The next step is to analyze the samples. Absorbance is measured and the concentration calculated from the standard curve.

High-Pressure Liquid Chromatography

Another method of analysis is chromatography. This is a separation technique based on the differing affinities of drug molecules between two phases. Various techniques such as thin-layer, column, liquid-liquid and gas chromatography are available. A relatively new method is high-pressure liquid chromatography (HPLC)[85,86]. The chromatographic column separates a mixture into its components and the UV spectrophotometer allows the quantitation of the components. The use of high pressure and small particles in the column allows for increased speed of separation and increased sensitivity.

The equipment required for isocratic HPLC includes a solvent delivery system which consists of a pump, solvent and tubing. The column is used to separate the mixture, a sensitive UV spectrophotometer measures transmittance

and a printer records the data (Figure V). The solvent, or mobile phase, is continuously pumped through the system. Isocratic analysis refers to the use of one concentration of the mobile phase. More than one pump and a system controller is used for gradient analyses, (the use of changing concentrations of the mobile phase). The sample is injected into the system via a loop injector and is carried with the mobile phase to the column. A precolumn is recommended to filter out contaminants in order to prolong the life of the separating column. The sample is separated in the column and then carried to the spectrophotometer. The transmittance (and absorbance) at the specified wavelength is measured and recorded on paper as a series of peaks for each component. This is the chromatogram. Quantitative analysis of the sample can be accomplished by measuring peak heights or area under the peaks. An integrator is often used to calculate the areas. Either measure correlates directly with the concentration.

The goal of HPLC is to separate a mixture such that the chromatogram gives peaks with an adequate degree of resolution. Resolution (or separation of the peaks) is a function of the capacity factor (k'), selectivity factor ($\propto$) and band spreading (N). The capacity factor is one way of calculating the retention time of injection, or the time where the two peaks elute relative to the time of injection. If the retention time is too long, then the time to do the analysis is increased. The selectivity factor describes where the peaks elute relative to one another. If the peaks elute at the same time, then there is no separation. If the peaks elute too close together, then there may be interference between the peaks and accurate analysis cannot be performed. Band spreading describes the height and width of the peaks. Tall, narrow peaks are more accurate than short, broad peaks. The latter may actually be a combination of two compounds and the chance of interference between the two compounds is increased.

The analysis of a variety of different compounds can be accomplished by changing the different variables in the system such as the type of column and the mobile phase. Column variables include the length and width of the column as well as the size, shape and chemical characteristics of the packing material. The mobile phase is usually described in terms of polarity index and the chemical group. An increase or decrease in the polarity can be accomplished by altering the concentrations of the mobile phase or changing to a different chemical group. Normal phase chromatography refers to the use of a non-polar solvent and polar column material while reverse phase refers to the use of a polar mobile phase and non-polar column.

SUMMARY

In summary, the availability of accurate stability data is crucial to ensure that patients are receiving effective and safe doses of medication. There are many variables that affect the stability of a drug and a change in one or more of these variables may change the rate of degradation. New stability data should be available for the use of antibiotics in sterile water for injection, in plastic syringes and at high concentrations. This information is not available and further study should be performed. This is necessary to ensure that the patient is administered a safe and effective dose when the drugs are administered in high concentrations by the syringe infusion pump system.

PREPARATION OF THE SOLUTIONS

The antibiotics used in this study were obtained in commercially available vials for reconstitution. They included sterile ampicillin sodium (Polycillin-N, Bristol) 2gm vials; sterile cefazolin sodium (Ancef, SKF) 1 and 10gm vials; sterile cefoxitin sodium (Mefoxin, MSD) 1,2 and 10gm vials; sterile piperacillin sodium (Pipracil, Lederle) 3 and 4gm vials; and sterile ticarcillin disodium (Ticar, Beecham) 3, 6 and 20gm vials. The water used to reconstitute the antibiotics was sterile water for injection (Travenol).

The concentrations of antibiotics were chosen to represent the most commonly used concentrations that are infused with the syringe-infusion pump system. Ampicillin sodium, cefazolin sodium and cefoxitin sodium were prepared at concentrations of 1gm/10ml and 2gm/10ml. Piperacillin sodium and ticarcillin disodium were prepared at concentrations of 2gm/10ml and 3gm/20ml. These solutions were prepared by reconstituting the appropriate number of vials with sterile water for injection. After the antibiotic was thoroughly mixed with a vortex mixer, the entire contents of each vial were withdrawn by syringe (Monoject plastic luer-lok with Monoject 1 inch 19 gauge needle) and combined in a volumetric flask. The concentration was adjusted with sterile water for injection and mixed thoroughly.

The syringes (12ml and 20ml Monoject plastic luer-lok) were filled immediately, capped with rubber luer-tip caps (Becton, Dickinson and Co.) and labeled. They were then placed in the appropriate storage area. Two syringes of each drug concentration were stored at room temperature ($24^{o}C$) and two each were refrigerated ($4^{o}C$). Six syringes of each drug and concentration were frozen ($-15^{o}C$).

ULTRAVIOLET SPECTROPHOTOMETRIC ANALYSIS

The ultraviolet spectrophotometric assays were to be done on a Beckman model 35 spectrophotometer. During the first few days, the absorbance readings could not be stabilized so an alternative method was designed. The spectrophotometer of an HPLC system was used. This system consisted of a Lambda-Max Model 480 LC spectrophotometer (Waters Associates) set at a sensitivity of 0.1 for ampicillin and 1.0 for the other antibiotics. A Model U6K Universal Liquid Chromatograph Injector (Waters Associates), a Model 6000A Solvent Delivery System (Waters Associates) and a Fisher Recordall Series 5000 printer were used. A column was not used. Samples were injected using a Hamilton microliter syringe #802. The mobile phase was double distilled water at a flow rate of 1ml/min. The wavelength was 260nm for ampicillin, cefazolin and cefoxitin, and 230nm for piperacillin and ticarcillin.

Standard curves (peak height vs. concentration) were prepared initially. Solutions of each antibiotic were prepared by reconstituting a vial of the antibiotic with sterile water for injection. The solution was mixed with a vortex mixer and the total contents withdrawn. This solution was further diluted to concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 0.6, 0.8 and 1.0mg/ml. Two samples of each concentration were injected. Peak heights were measured and the average for each concentration was plotted on linear graph paper. The line of the standard curve was determined by linear regression analysis.

Within one hour of the preparation of the solutions in the syringes, a sample was taken to be assayed for initial concentration. Thereafter, a sample was taken from the room temperature and refrigerated syringes at the appropriate time. These syringes were returned to the storage temperature immediately. Syringes removed from the freezer were thawed at room temperature for two hours and

assayed. These syringes were not refrozen but were kept in the refrigerator for later use.

Room temperature and refrigerated samples were assayed at 12 hours and then on days 1, 2, 3, 4, 7, 14 and 28. Frozen syringes were assayed on days 28, 56 and 84. In addition, ampicillin syringes at room temperature and under refrigeration were assayed at hours 4 and 8. It was suspected that this antibiotic would degrade much faster than the others based on previous studies.

In order to assay the samples from the syringes, the samples had to be diluted. All samples were diluted with double distilled water (1:400 for 2gm/10ml, 1:200 for 1gm/10ml and 1:300 for 3gm/20ml). After mixing thoroughly, a 3 microliter sample (10 microliters for ampicillin) was injected 3 times. Thus for each antibiotic concentration, two syringes were prepared. From each syringe, two dilutions were made and from each dilution, three samples were injected for a total of 12 injections for each drug concentration and sample time. Peak heights were measured and the average with standard deviation was calculated. Peak heights were then converted to concentrations using the standard curve.

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS

An HPLC analysis of these solutions was developed for all antibiotics except for cefoxitin. There was sufficient data in the literature indicating that UV spectrophotometry of cefoxitin was stability-indicating[74-76]. The other four antibiotics were analyzed with HPLC to determine whether the degradation products absorbed UV light at the same wavelengths as the parent compounds.

The HPLC assay was performed on the same equipment as the previous assays except for the addition of a column, Waters microbondapak, 3.9mm x 30cm (#27324).

Preliminary Studies

Piperacillin was prepared as a fresh solution at a concentration of 0.5mg/ml. Samples from the syringes left over from the previous work were used to identify the degradation products. The initial mobile phase used was 22% acetonitrile in 0.1M sodium acetate buffer, with a pH of 4.6 adjusted with glacial acetic acid[87]. The wavelength was set at 254nm and then 230nm. The UV absorbance was much too high at 254nm such that the spectrophotometer could not be calibrated. At 230nm, there were no peaks. Another mobile phase was tried. This consisted of methanol (450ml), 0.2M monobasic sodium phosphate (100ml) and tetrabutylammonium hydroxide solution (3ml of 1:10 solution in water) and double distilled water (qs to 1 liter). The pH was adjusted to 5.5 with phosphoric acid and the final solution was degassed[88]. The mobile phase was used at a flow rate of 1ml/min and the spectrophotometer was set at a wavelength of 230nm. Resolution of the peaks was very good. The volume injected was 25 microliters.

Ticarcillin was prepared in the same manner as piperacillin. The mobile phase that was used initially was the methanol-monobasic sodium phosphate solution that was used with piperacillin. The peaks from ticarcillin were very narrow but the selectivity factor was poor. The flow rate was decreased to 0.5ml/min but this did not help significantly. Then the mobile phase was diluted 50:50 with double-distilled water. This was used at a flow rate of 0.5ml/min and then 1.0ml/min. The faster flow rate was used for the study.

Cefazolin was prepared in the same manner and injected with the mobile phase that was used for piperacillin. The wavelength was 260nm. Peaks were well separated, no other changes were made.

The only modifications for ampicillin was to increase the sensitivity to 0.1 and the wavelength to 260nm. The volume injected was 19 microliters.

HPLC Analysis

HPLC analysis of ampicillin, cefazolin, piperacillin and ticarcillin was performed. The HPLC assay used for each drug is summarized in Table VI. A fresh solution for each drug (0.5mg/ml) in double distilled water was prepared. Two 25 microliter samples of each (10 microliters for ampicillin) were injected.

Frozen samples (2gm/10ml) were thawed at room temperature for two hours. Samples were diluted 1:400 with double-distilled water and then injected.

The syringes that were used at room temperature and under refrigeration were also assayed. In addition, the frozen samples that were thawed and then refrigerated were also assayed.

The piperacillin and ticarcillin samples consisted of the fresh solution, room temperature (3 months), refrigerated (3 months), frozen and then refrigerated (1, 2 and 3 months) and recently thawed samples. All concentrations used were 2gm/10ml. These were diluted 1:400 and assayed.

Ampicillin and cefazolin samples were the same except that the room temperature samples were not assayed. These solutions were a very dark amber color, probably due to the degradation products or to contamination. Analysis of these solutions would be inconclusive.

Peak heights were measured and the standard curve for each drug was adjusted using the ratio of peak height with the column to the peak height without the column. The concentrations of the samples were calculated from the standard curve and plotted on a concentration-time graph.

ANALYSIS OF THE DATA

The concentration of each antibiotic was plotted against time. Since these antibiotics degrade by a first order rate process, the first order rate equation for this graph was calculated via linear regression analysis. The rate constant was used to determine the recommended expiration date of the solutions. The time to degrade by 10% was used to determine the stability.

In addition to the rate equations, the rate constants and the temperatures were used to generate the Arrhenius equation for each antibiotic.

AMPICILLIN

The standard curve for ampicillin is shown in Figure VI. The peak height varies directly with concentration between 0.025 and 1.0mg/ml. The correlation coefficient was 99.7%. The data for concentration and time is shown in Figures VII and VIII. HPLC chromatograms (Figure IX) showed that the degradation products absorb UV light at 260nm and that multiple products are formed. Kinetic analysis of the UV data was not possible due to interference by the degradation products.

CEFAZOLIN

The standard curve for cefazolin is shown in Figure X. Peak heights varied directly with concentration between 0.025 and 0.5mg/ml. The correlation coefficient was 99.7%. Figures XI and XII are the graphs of concentration versus time. Data plotted on semi-log paper were linear, indicating a pseudo-first order process. The correlation coefficients were 89.5% for room temperature solutions of 1gm/10ml and 91.5% for the 2gm/10ml solutions.

There was no change in concentration in the frozen syringes over 3 months. Refrigerated samples of cefazolin showed minimal change. The rate constant for room temperature samples was 0.0067 days^{-1} and 0.0079 days^{-1} for 1gm and 2gm/10ml samples, respectively (Table VII). The room temperature samples were stable for 13 days, refrigerated samples were stable up to 30 days and frozen samples were stable up to 3 months. The HPLC analysis of fresh, frozen and refrigerated solution showed no interference by the degradation products. (Figure VIII).

The relationship between rate constant for degradation and temperature is:

$$\log k = 38 - 11937\left(\frac{1}{T}\right).$$

CEFOXITIN

The standard curve for cefoxitin is shown in Figure XIV. Peak heights and concentration are directly proportional between 0.025 and 0.5mg/ml. The correlation coefficient is 99.9%. The concentration-time curve is shown in Figure XV and XVI. The data shows that cefoxitin follows a pseudo-first order degradation rate process. The rate constant for room temperature samples was 0.32 days^{-1} (97.1% correlation coefficient) and 0.052 days^{-1} (96.4%) for 1 and 2gm syringes, respectively. The rate constant for refrigerated samples was 0.0045 days^{-1} (71.5%) and 0.004 days^{-1} (85.3%) for 1 and 2gm syringes, respectively. There was no change in the concentration of the frozen syringes. The time to degrade by 10% for the room temperature samples was 2 days and the time for 10% degradation for refrigerated samples was 23 days. Frozen samples were stable up to 3 months. The relationship between the degradation rate constant and temperature is:

$$\log k = 10.3 - 3516(\tfrac{1}{T}).$$

PIPERACILLIN

The standard curve for piperacillin is shown in Figure XVII. There is a linear relationship between peak heights and concentration between 0.025 and 0.6mg/ml. The correlation coefficient was 99.7%. The data using UV spectrophotometry is shown in Figures XVIII and XIX. There appears to be no change in concentration over the period studied. Subsequent analysis by HPLC showed several degradation products absorbing at 230nm (Figure XX). Figure XXI is the concentration-time graph based on HPLC analysis. Analysis of the HPLC data gave reaction rate constants for degradation of 0.44 days^{-1} and 0.01 days^{-1} for room temperature and refrigerated temperatures respectively (Table V). Frozen samples showed no change over 3 months. The time for 10% loss of concentration at room temper-

ature was 2 days and under refrigeration it was 10 days. The relationship between the degradation rate constant and temperature was:

$$\log k = 7.56 - 2652(\tfrac{1}{T}).$$

TICARCILLIN

The standard curve for ticarcillin is shown in Figure XXII. Peak heights and concentration were directly proportional between 0.025 and 0.6mg/ml. The correlation coefficient was 99.6%. The data from UV spectrophotometry is shown in Figures XXIII and XXIV. As with piperacillin, HPLC analysis showed that the degradation products interfere with the analysis of the parent compound (Figure XXV). Figure XXVI shows the concentration-time graph of ticarcillin using HPLC. This data was used to calculate the following rate constants of degradation: 0.022 days^{-1} for room temperature and 0.017 days^{-1} for refrigerated samples. Frozen solutions did not change after 3 months. Room temperature samples were stable for 4 days and refrigerated samples were stable for 6 days. The relation between degradation rate constant and temperature is:

$$\log k = -.107 - 461(\tfrac{1}{T}).$$

DISCUSSION

AMPICILLIN

The ultraviolet absorption by ampicillin appeared to increase over time (Figure VII). Since absorption and concentration vary directly, one would expect the absorption of ampicillin to decrease over time. Raffanti and King[51] published UV scans of ampicillin and showed absorbance to increase or decrease depending on the pH of the solution. The sample pH was not measured during this study. The pH of the solution could have changed resulting in an increase in absorbance of the solution. The absorbance could also increase due to interference by the degradation products. HPLC analysis showed several degradation products that absorb at 260nm. The absorbance by these products could account for the lack of change in peak heights for refrigerated, room temperature and frozen samples. Degradation rate constants were not calculated due to these two problems.

The purpose of freezing these antibiotics is to decrease the rate of degradation. Many pharmacists can increase the efficiency of their admixture service by preparing these solutions in quantity, freezing them and then thawing them as needed. As other investigators reported[57,58], frozen solutions of ampicillin degraded even at temperatures of -10°C to -20°C. Loss of 10% of the parent compound can occur within the first 24 hours at -20°C[58]. Even though UV absorbance did not change, the HPLC chromatograms showed that ampicillin in sterile water for injection did degrade by 93% in 3 months. Thus, even in sterile water for injection, ampicillin solutions degrade very rapidly at -15°C. Therefore, the benefit of freezing these solutions is lost.

CEFAZOLIN

Cephalosporin degradation products are a result of rapid hydrolysis of the beta-lactam ring and subsequent breakdown to multiple products. These products

do not absorb UV light at 260nm. HPLC analysis confirmed this (Figure VIII). A single peak representing cefazolin appeared for each sample tested. Thus, UV analysis data represented loss of parent compound.

Frozen solutions were stable up to 3 months. Previous studies also confirmed that cefazolin solutions (NS or D5W) could be frozen up to 1 month[54], 3 months[71], 7 months[71] and 9 months[72].

Refrigerated solutions were stable for 30 days. There was no change in the concentration during the period studied. Previous studies at other concentrations and solutions showed stability up to 7 days[70], 14 days[70], 15 days[63] and 24 days[63]. Samples at room temperature were stable for 13 days. Previous studies reported stability up to 4 days[70], 5 days[63] and 7 days[70]. These differences could be due to the assays and methods of analysis. It could also be due to the difference in stability between sterile water for injection and saline or dextrose. Most of the shorter expiration dates were obtained from studies in saline and dextrose.

CEFOXITIN

Cefoxitin is similar to cefazolin in that direct UV spectrophotometry is comparable to HPLC analysis.

Frozen solutions were stable for up to 3 months. One previous study of various concentrations showed that these solutions were stable up to 30 weeks (7 months)[75,77].

Refrigerated samples were stable for 23 days. Previous studies showed stability up to 7 days with UV analysis[75,77] and up to 13-44 days with HPLC[78].

Variations could be due to the methods of analysis of the data. Many studies base expiration dates by calculating per cent loss of concentration at different analysis times. For instance, Gupta and Stewart[78] assayed their samples on day 13 and then on day 44. Their conclusion (expiration date of 13 days) were based on one assay on day 44, which showed 89% of original concentration. Any variation in the assay could alter the results. Analysis of the data by calculating the degradation rate takes into account several data points and does not rely on one point or even two points. Thus, there is less chance of error.

Room temperature samples were stable for up to 2 days. This was comparable to other studies reporting dates of one to two days[72,74,75].

PIPERACILLIN

Direct ultraviolet spectrophotometric analysis of piperacillin was not specific for the parent compound. The UV data showed little change in absorbance of the solution over time. In contrast, HPLC chromatograms showed several other peaks absorbing at 230nm, representing the degradation products. The more concentrated samples (2gm/10ml) were chosen to be analyzed using HPLC. The rate constant from these samples was calculated.

The manufacturers' data covers a period of only one month. Our results indicated that frozen solutions were stable for up to 3 months. The chromatogram of this solution had one peak, that of piperacillin, and no peaks from degradation products.

Refrigerated samples were stable up to 10 days. The manufacturer recommended 2 days to one week[61,62]. Room temperature samples were stable for up to 2 days. The manufacturer recommends 1-2 days[61,62]. The manufacturers'

recommendations for expiration dating is shorter than the expiration date from this analysis. One consideration that the manufacturer may have taken into account for the shorter expiration date is bacterial contamination and growth that may occur during preparation of the solutions. The use of sterile technique when preparing these solutions may eliminate the need for this strict expiration dating.

TICARCILLIN

Analysis of ticarcillin was similar to piperacillin. UV spectrophotometric data was not used because the degradation products appeared on the HPLC chromatogram. Ticarcillin appears as two peaks representing the D- and L-isomers. Both degrade at the same rate[63].

Frozen solutions were stable for 3 months based on HPLC analysis. No degradation products appeared on the chromatogram. The manufacturers' data is limited to 30 days[65].

Refrigerated solutions were stable for 6 days. Other studies reported stability of NS and D5W solutions for 3 days[65], 7 days[64] and 14 days[65] based on microbiologic analysis. HPLC analysis showed stability up to 15 days[63]. Room temperature solutions were stable up to 4 days. Other studies reported 1 day[64,65] and 3 days[65]. Again, these differences could be due to analysis technique and the assay used.

GENERAL COMMENTS

Previous studies[20,66,74], as well as this study, showed that these beta-lactam antibiotics degrade by a pseudo-first order process. Concentration-time data is linear on semi-log paper, indicating that the rate of degradation varies with the concentration of the drug.

Containers should not affect stability unless sorption occurs. Sorption should not occur because these compounds are very hydrophilic. Leaching was not studied. Leaching from rubber or plastic may be possible but the significance is questionable based on studies with similar solutions[30,35,39].

The solvent may play a role in altering the rate of degradation. The rates are generally faster in dextrose solutions than in normal saline or water. Formation of polymers in these solutions was not studied.

UV spectrophotometry is acceptable for cephalosporins such as cefazolin and cefoxitin, but not for the penicillins. Penicillins have multiple degradation products that have an intact beta-lactam ring and absorb UV light. Microbiologic assays may not be specific either if the degradation products have some antibacterial activity. Chemical assays such as HPLC are necessary to study these antibiotics.

Published data are incomplete. Many studies look at per cent change in concentration at specific times. Decisions concerning expiration dates are then based on one data point. A kinetic study using degradation rate constants is rarely done. This is a more accurate method due to the use of more data points, increasing the reliability of the results. The analysis in this study had as many as 10 points to determine the degradation rate constants and each point was based on the average of 12 assays. The standard deviations of the averaged concentrations (1 or 2 gms/10-20ml) was usually $\pm$ .01-.1%.

The methods of analysis used in this study were extremely accurate. UV spectrophotometry and HPLC are highly sensitive and specific methods of analysis. In addition, the use of 12 assays for each sample eliminates any error in

dilutions and injection technique. The very low standard deviations show that the results are reliable. In addition, the very high correlation coefficients for the concentration-time profiles support this.

The temperature-rate constant equations were developed in order to determine the rate of degradation at any temperature (Table VIII). Room temperature in actual practice is not constant at 24°C-25°C but may vary with geographic location and time of the year. Pharmacy policy may state that all intravenous admixtures should be refrigerated if not used within one hour. The situation often arises where an IV preparation is unintentionally left at room temperature. If the preparation was ampicillin, the admixture should be discarded because this drug has a much faster degradation rate at room temperature. If the drug was cefazolin, then the preparation could still be used since this drug has almost the same degradation rate at room temperature than under refrigeration. Thus, the knowledge of the effect of temperature on the reaction rate constant could help in making these decisions.

SUMMARY AND CONCLUSIONS

The syringe-infusion pump system is a new, alternative method for administering intravenous antibiotics. Before implementation of this system, pharmacists must know the stability of these medications when prepared at high concentrations and stored in disposable plastic syringes. The pharmacist must ensure that the doses prepared contain at least 90% of the labeled concentration and that degradation products are not toxic. In addition, to use this system efficiently, expiration times should be known so that unused syringes can be either discarded or returned to storage. This information is not available in the pharmacy literature. The following is a summary of the conclusions of this study:

1. Ampicillin solutions lost 93% of the initial concentration at 3 months when stored at -15°C.

2. Cefazolin is stable at room temperature for 13 days, when refrigerated for 30 days and frozen for 3 months.

3. Cefoxitin is stable at room temperature for 2 days, when refrigerated for 23 days and frozen for 3 months.

4. Piperacillin is stable at room temperature for 2 days, refrigerated for 10 days and frozen for 3 months.

5. Ticarcillin is stable at room temperature for 4 days, when refrigerated for 6 days and frozen for 3 months.

6. Ampicillin, ticarcillin and piperacillin cannot be assayed by direct ultra-violet spectrophotometry due to interference by degradation products. HPLC is a preferred method.

7. Cefazolin and Cefoxitin can be assayed by direct ultraviolet spectrophotmetry since the degradation products do not absorb at 260nm.

8. Rate constant and temperature equations are ideal to summarize the influence of temperature on degradation rates. Once the equation is determined, then the time to degrade by 10% can be calculated at any temperature.

All of the antibiotics studied, except ampicillin, have adequate stability for use in this system. These four antibiotics were stable for at least 2 days at room temperature and at least 6 days when refrigerated. These medications, once prepared, should still be refrigerated as soon as possible to prolong storage time. However, if they are unintentionally left at room temperature, they still can be used up to 2 days. The longer storage time for refrigerated solutions enables the pharmacist to use these up to 6 days after preparation. Further study with ampicillin must be performed before accurate stability data can be given.

All antibiotics, except ampicillin, were stable when frozen for 3 months. This gives pharmacy admixture services, with high workloads, the option of freezing these medications for later use. Again, the exception is ampicillin, which still degrades significantly at temperatures of -15°C.

The use of these 4 beta-lactam antibiotics in the syringe infusion pump system is an option that pharmacists can use in their admixture services.

TABLE I
SUMMARY OF STABILITY DATA OF INTRAVENOUS AMPICILLIN SOLUTIONS

Temperature	Concentration	Solvent	Container	Assay	T_{90}[a]	Source
RT[b]	Up to 3%	SWI/NS[c]	--	--	8 hrs	6
RT	1-2%	D5W[e]	--	--	2 hrs	6
RT	2%	NS	--	micro	8 hrs	60
RT	2%	D5W	--	micro	30 min	60
RT	2%	NS	PVC[e]/glass	micro	8 hrs	7
RT	2%	D5W	PVC/glass	micro	<4 hrs	7
RF[f]	Up to 3%	SWI/NS	--	--	48 hrs	6
RF	1-2%	D5W	--	--	4 hrs	6
RF	2%	SWI/NS	--	--	72 hrs	6
RF	2%	NS	--	micro	24 hrs	60
RF	2%	D5W	--	micro	8 hrs	60
RF	2%	NS	PVC/glass	micro	24 hrs	7
RF	2%	D5W	PVC/glass	micro	8 hrs	7
F[g](-30°C,-70°C)	2%	NS	PVC	micro	30 days	57
F (-70°C)	2%	D5W	PVC	micro	30 days	57
F (-20°C)	2%	NS	PVC	micro	1 day	58
F (-20°C)	2%	D5W	PVC	micro	<1 day	58

a Time for 10% loss of initial concentration or 10% loss of activity.

b RT = room temperature.

c SWI = sterile water for injection NS = normal saline.

d D5W = 5% dextrose in water.

e PVC = polyvinyl chloride bags.

f RF = refrigeration (4°C to 6°C).

g F = frozen (-10°C to -20°C).

TABLE II
SUMMARY OF STABILITY DATA OF INTRAVENOUS PIPERACILLIN SOLUTIONS

Temperature	Concentration	Solvent	Container	Assay	$T_{90}{}^a$	Source
RT[b]	--	NS/D5W/SWI[c]	PVC[d]/glass	--	24 hrs	61
RT	12%	NS/D5W	glass	--	24 hrs	62
RT	0.2%	NS/D5W	PVC/glass	--	24 hrs	62
RF[e]	--	NS/D5W/SWI	PVC/glass	--	1 week	61
RF	12%	NS/D5W	glass	--	1 week	62
RF	0.2%	NS/D5W	PVC/glass	--	48 hrs	62
F[f]	--	NS/D5W/SWI	PVC/glass	--	1 month	61
F	0.2%	NS/D5W	PVC/glass	--	1 month	62
F	40%	SWI	glass/plastic syringe	--	32 days	62
F	12%	NS/D5W	glass	--	32 days	62

a Time for 10% loss of initial concentration or 10% loss of activity.

b RT = room temperature.

c NS = normal saline, D5W = 5% dextrose in water, SWI = sterile water for injection.

d PVC = polyvinyl chloride bags.

e RF = refrigeration (4°C to 6°C).

f F = frozen (-10°C to -20°C).

TABLE III
SUMMARY OF STABILITY DATA OF INTRAVENOUS TICARCILLIN SOLUTIONS

Temperature	Concentration	Solvent	Container	Assay	T_{90}[a]	Source
RT[b]	500mg/ml	SWI[c]	--	micro	1 day	64
RT	1-5-10%	NS/D5W/SWI[d]	--	--	3 days	65
RT	2%	NS/D5W	--	micro	1 day	64
RT	2%	NS/D5W	PVC[e]	HPLC	1 day	63
RF[f]	500mg/ml	SWI	--	micro	7 days	64
RF	1-5-10%	NS/D5W/SWI	--	--	14 days	65
RF	2%	NS/D5W	PVC	HPLC	15 days	63
F[g]	1-5-10%	NS/D5W/SWI	--	--	30 days	65

a Time for 10% loss of initial concentration or 10% loss of activity.

b RT = room temperature.

c SWI = sterile water for injection.

d NS = normal saline, D5W = 5% dextrose in water.

e PVC = polyvinyl chloride bags.

f RF = refrigeration (4°C to 6°C).

g F = frozen (-10°C to -20°C).

TABLE IV
SUMMARY OF STABILITY DATA OF INTRAVENOUS CEFAZOLIN SOLUTIONS

Temperature	Concentration	Solvent	Container	Assay	T_{90}[a]	Source
RT[b]	—	NS/D5W/SWI[c]	—	—	24 hrs	68, 69
RT	0.5%	NS	glass	micro	7 days	70
RT	0.5%	D5W	glass	micro	4 days	70
RT	2%	NS/D5W	glass	HPLC	5 days	63
RT	2%	NS/D5W	PVC/glass	micro	24 hrs	7
RT	1gm/4ml	SWI	glass	micro	4 days	70
RF[d]	—	NS/D5W/SWI	—	—	96 hrs	68, 69
RF	0.5%	NS	glass	micro	7 days	70
RF	0.5%	D5W	glass	micro	14 days	70
RF	2%	NS/D5W	glass	HPLC	15 days	63
RF	2%	NS/D5W	PVC/glass	micro	24 hrs	7
RF	1gm/4ml	SWI	glass	micro	14 days	70
F[e]	1gm/2.5ml	NS/D5W/SWI	glass	micro	26 wks	71
F	1gm/3ml	SWI	plastic syringe	micro	9 months	72
F	2%	NS/D5W	PVC	micro	30 days	58
F	0.5%	D5W	glass	micro	26 wks	71
F	0.5%	NS/SWI	glass	micro	12 wks	71
F	—	NS/D5W/SWI	original	—	12 wks	69

a Time for 10% loss of initial concentration or 10% loss of activity.

b RT = room temperature.

c NS = normal saline, D5W = 5% dextrose in water, SWI = sterile water for injection.

d RF = refrigeration (4°C to 6°C).

e F = frozen (-10°C to -20°C).

TABLE V
SUMMARY OF STABILITY DATA OF INTRAVENOUS CEFOXITIN SOLUTIONS

Temperature	Concentration	Solvent	Container	Assay	T_{90}[a]	Source
RT	0.1-2%	NS/D5W/SWI[b]	—	—	24 hrs	77
RT	0.5,1%	NS/D5W	glass	UV	24 hrs	75
RT	2%	NS/D5W	PVC	HPLC	24 hrs	78
RT	10%	NS/D5W/SWI	original	—	24 hrs	77
RT	10%	SWI	plastic syringe	UV	24 hrs	75
RF[d]	0.1-2%	NS/D5W/SWI	—	—	48 hrs	77
RF	0.5,1%	NS	glass	UV	48 hrs[e]	75
RF	0.5,1%	D5W	glass	UV	7 days	75
RF	2%	NS/D5W	PVC	HPLC	13 days[f]	78
RF	10%	NS/D5W/SWI	original	—	7 days	77
F[g]	10%	NS/D5W/SWI	glass	UV	30 wks	75
F	10%	NS/D5W/SWI	original	—	30 wks	77

a Time for 10% loss of initial concentration or 10% loss of activity.

b NS = normal saline, D5W = 5% dextrose in water, SWI = sterile water for injection.

c PVC = polyvinly chloride bags.

d RF = refrigeration (4°C to 6°C).

e limited the study to 48 hours.

f 95% concentration left at 13 days and 89% left at 44 days.

g F =frozen (-10°C to -20°C).

TABLE VI   HPLC ASSAY CONDITIONS FOR THIS STUDY

Antibiotic	Mobile Phase*	Flow Rate	Sensitivity	Wavelength	Injection Volume	Paper Speed
Piperacillin	A	1ml/min	1.0	230	25ml	.2in/min
Ticarcillin	B	1ml/min	1.0	230	25ml	.2in/min
Cefazolin	A	1ml/min	1.0	260	25ml	.2in/min
Ampicillin	A	1ml/min	0.1	260	10ml	.2in/min

* Sol A = 450ml methanol, 100ml monobasic sodium phosphate, 3ml tetrabutylammonium hydroxide (1:10) qs 1L with double distilled H_2O. Adjust pH to 5.5 with phosphoric acid.

Sol B = 50:50 Solution A and double distilled H_2O.

TABLE VII
Pseudo-first-order degradation rate constants and calculated times to degrade by
10% (t90), from this study.

Drug	Temperature	k (2gm/10ml)	Time to lose 10%*	
Cefazolin	24°C	0.0079 days^{-1}	13	days
	4°C	0	30	days
	-15°C	0	3	months
Cefoxitin	24°C	0.052 days^{-1}	2	days
	4°C	0.0038 days^{-1}	23	days
	-15°C	0	3	months
Piperacillin	24°C	0.044 days^{-1}	2	days
	4°C	0.01 days^{-1}	10	days
	-15°C	0	3	months
Ticarcillin	24°C	0.022 days^{-1}	4	days
	4°C	0.017 days^{-1}	6	days
	-15°C	0	3	months

* t90 calculated from $\log C = \log C_o - \frac{kt}{2.303}$ or $t_{90} = \frac{.104}{k}$.

TABLE VIII Arrhenius Equation for antibiotics studied. Rate constant (k) versus Temperature (^{o} Kelvin).

Cefazolin $\qquad\qquad$ log k = 38.0 - 11937 $(\frac{1}{T})$

Cefoxitin $\qquad\qquad$ log k = 10.3 - 3516 $(\frac{1}{T})$

Piperacillin $\qquad\quad$ log k = 7.56 - 2652 $(\frac{1}{T})$

Ticarcillin $\qquad\quad$ log k = -0.107 - 461 $(\frac{1}{T})$
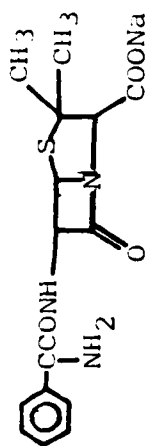
Cefazolin

Cefoxitin

Ampicillin

Piperacillin
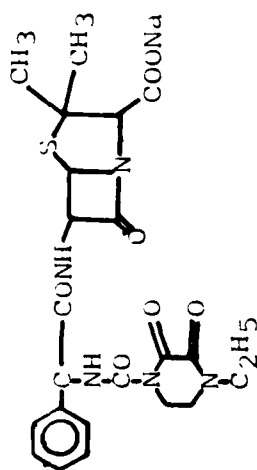
Ticarcillin
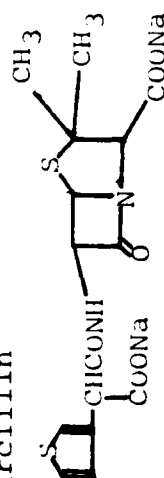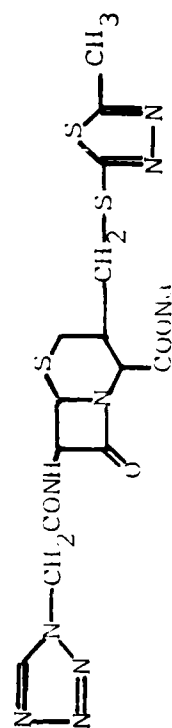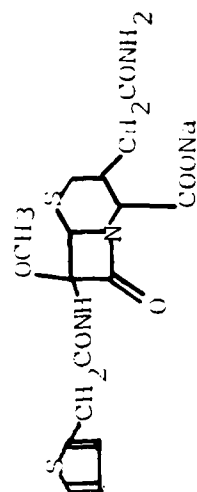
Figure 1   Structures of the beta-lactam antibiotics studied.
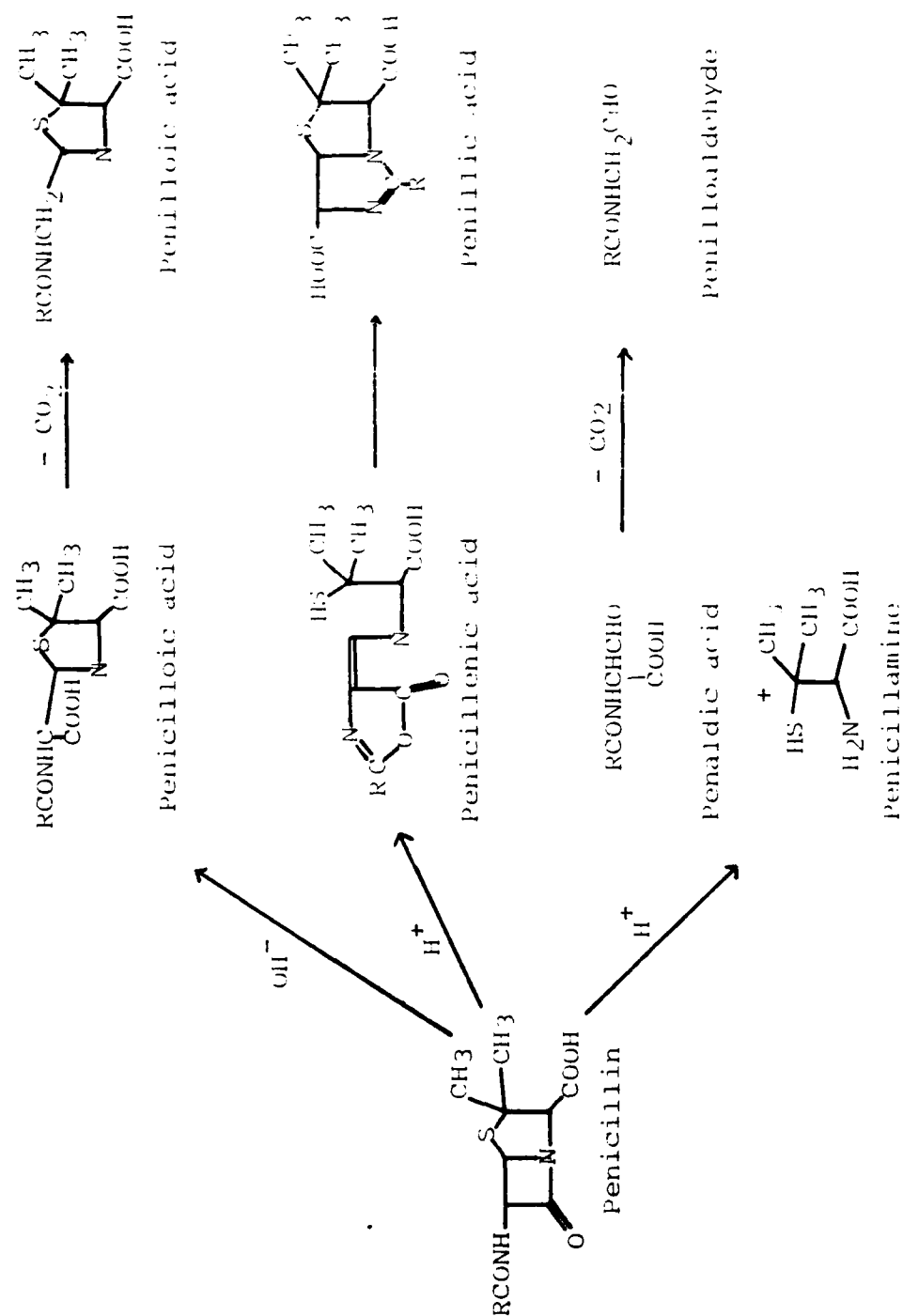
Figure 11 Penicillin degradation products (adapted from references 26,42,48).

Alpha-aminobenzylpenilloic
acid

CNH₂CONHCH₂

$-CO_2$

Alpha-aminobenzylpenicilloic
acid

Alpha-aminobenzylpenicillinic acid

OH⁻

H⁺

Alpha-aminobenzylpenamaldic acid

H⁺

Ampicillin

Alpha-aminobenzylpenillic acid
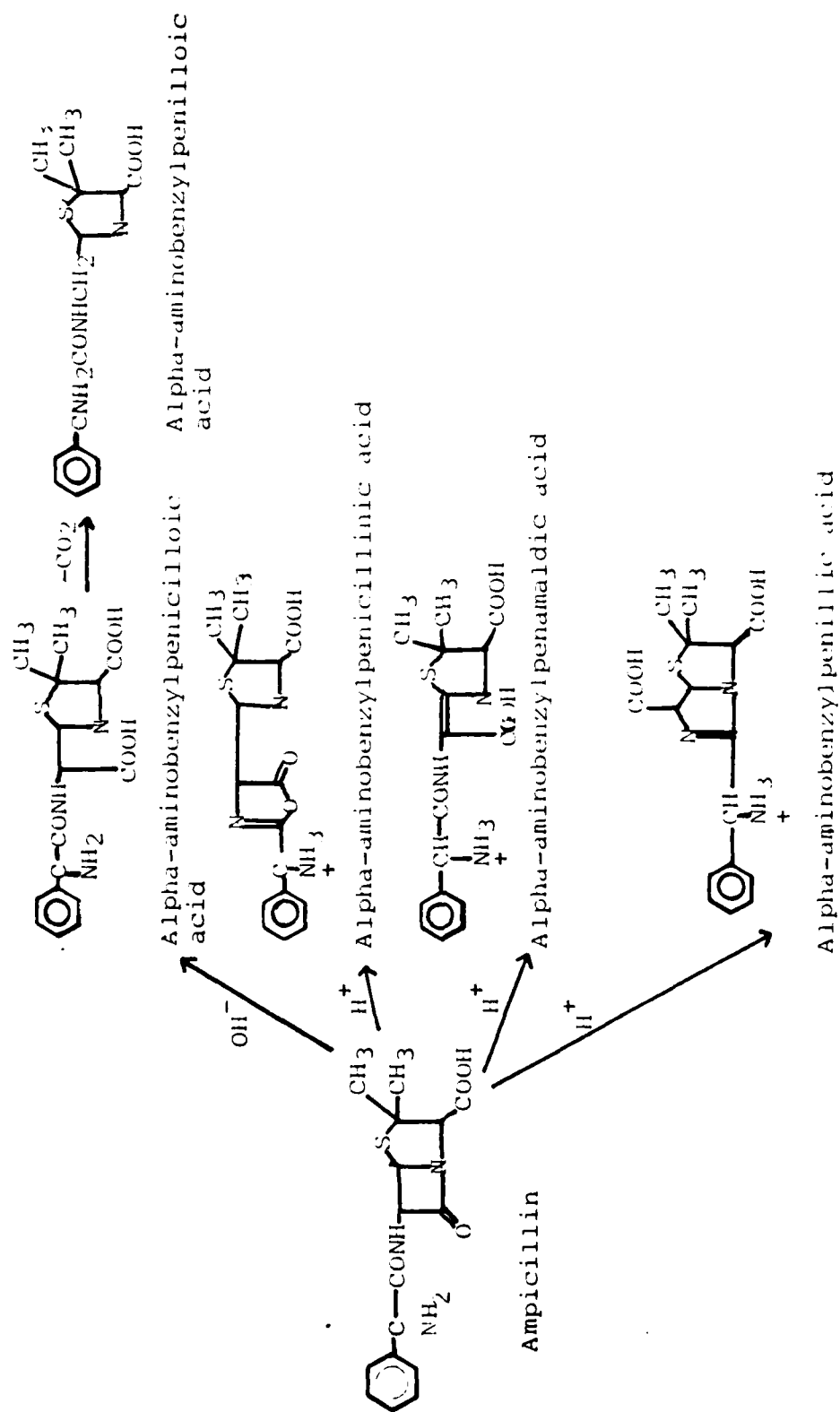
Figure III   Ampicillin degradation products (reference 25).
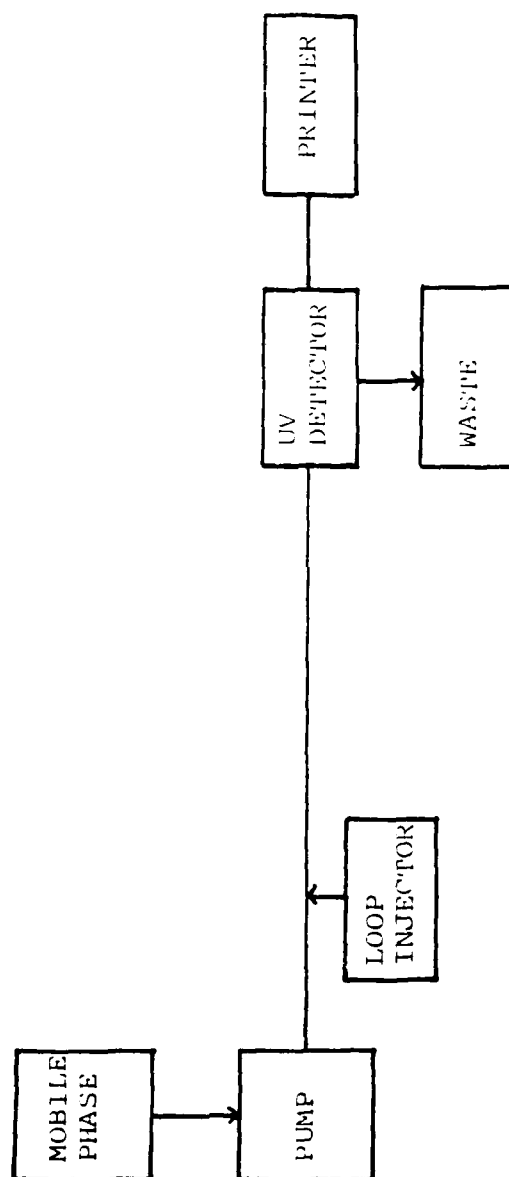
Figure IV   Diagram of UV spectrophotometer used in this study.
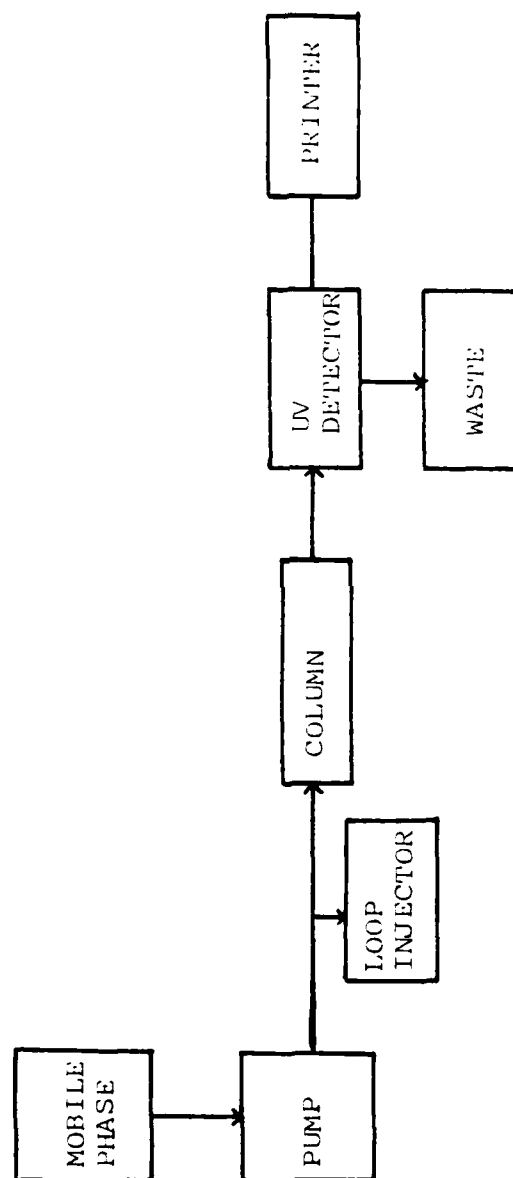
Figure V   Diagram of isocratic HPLC used in this study.
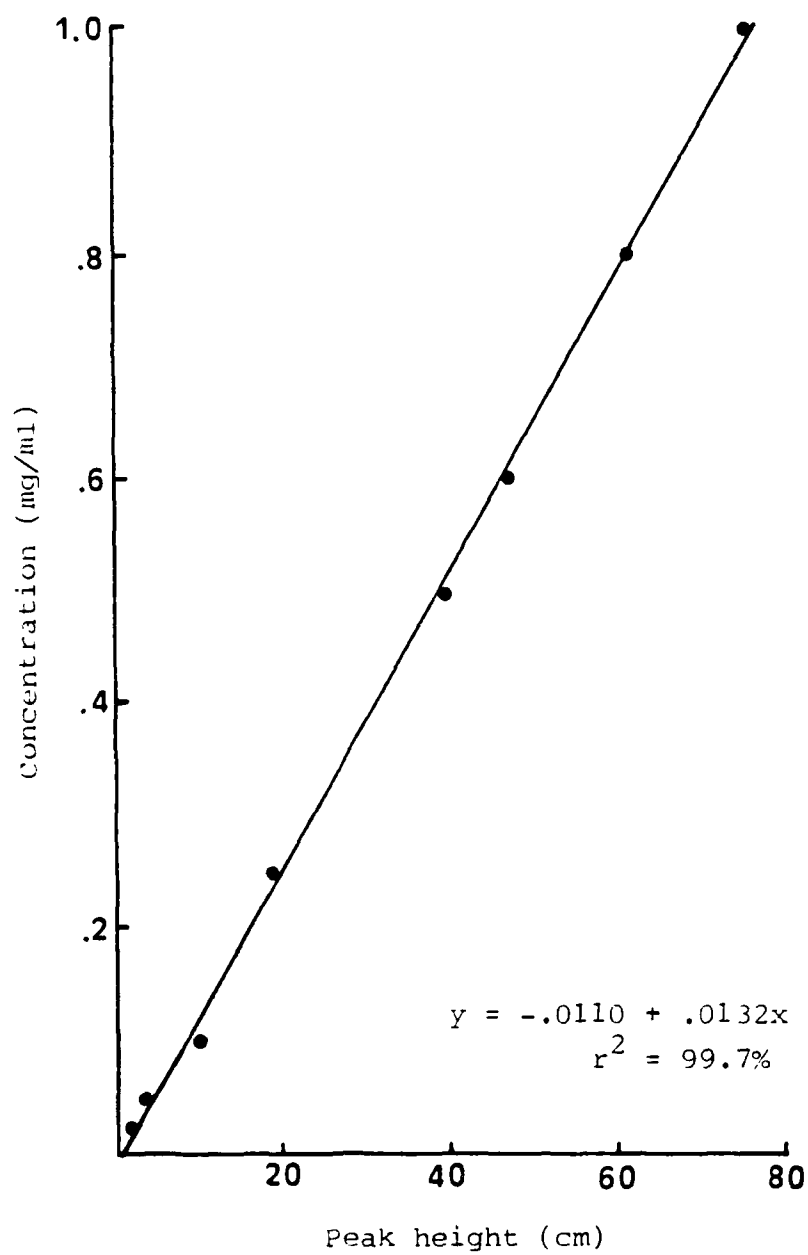
Figure VI Ampicillin Standard Curve
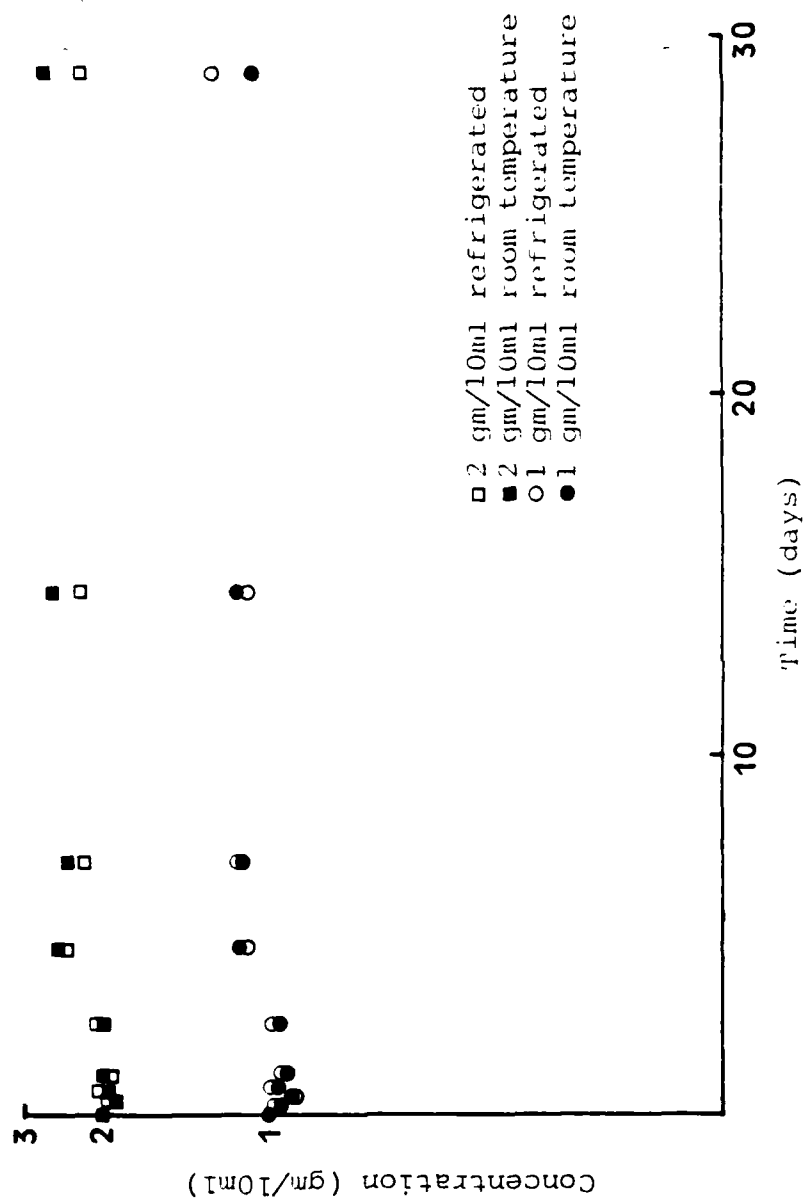
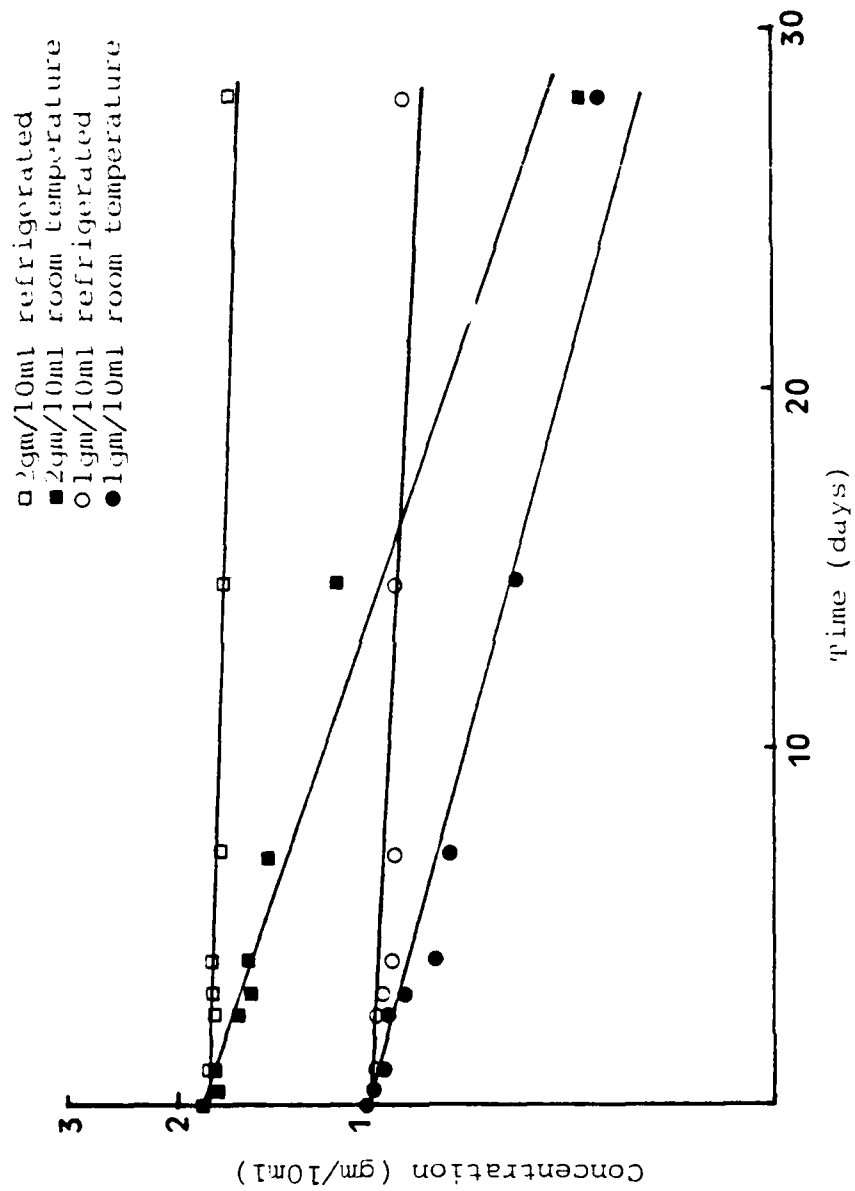

Figure VII Concentration-time profile of ampicillin, using UV analysis.
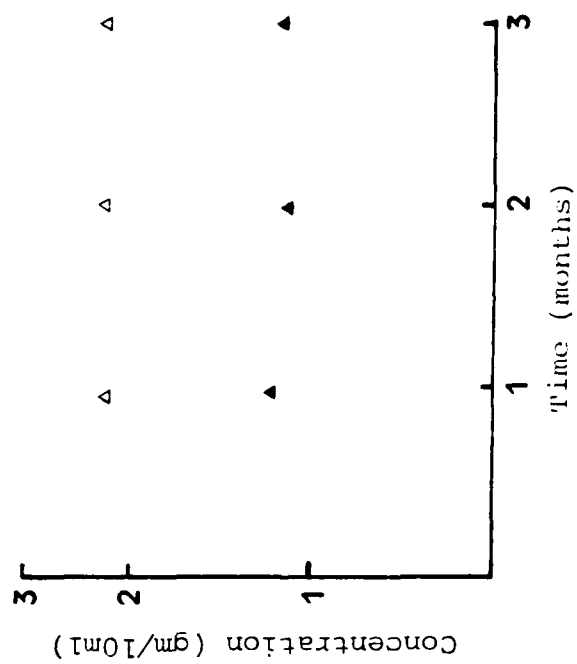
Figure VIII Concentration-time profile of ampicillin
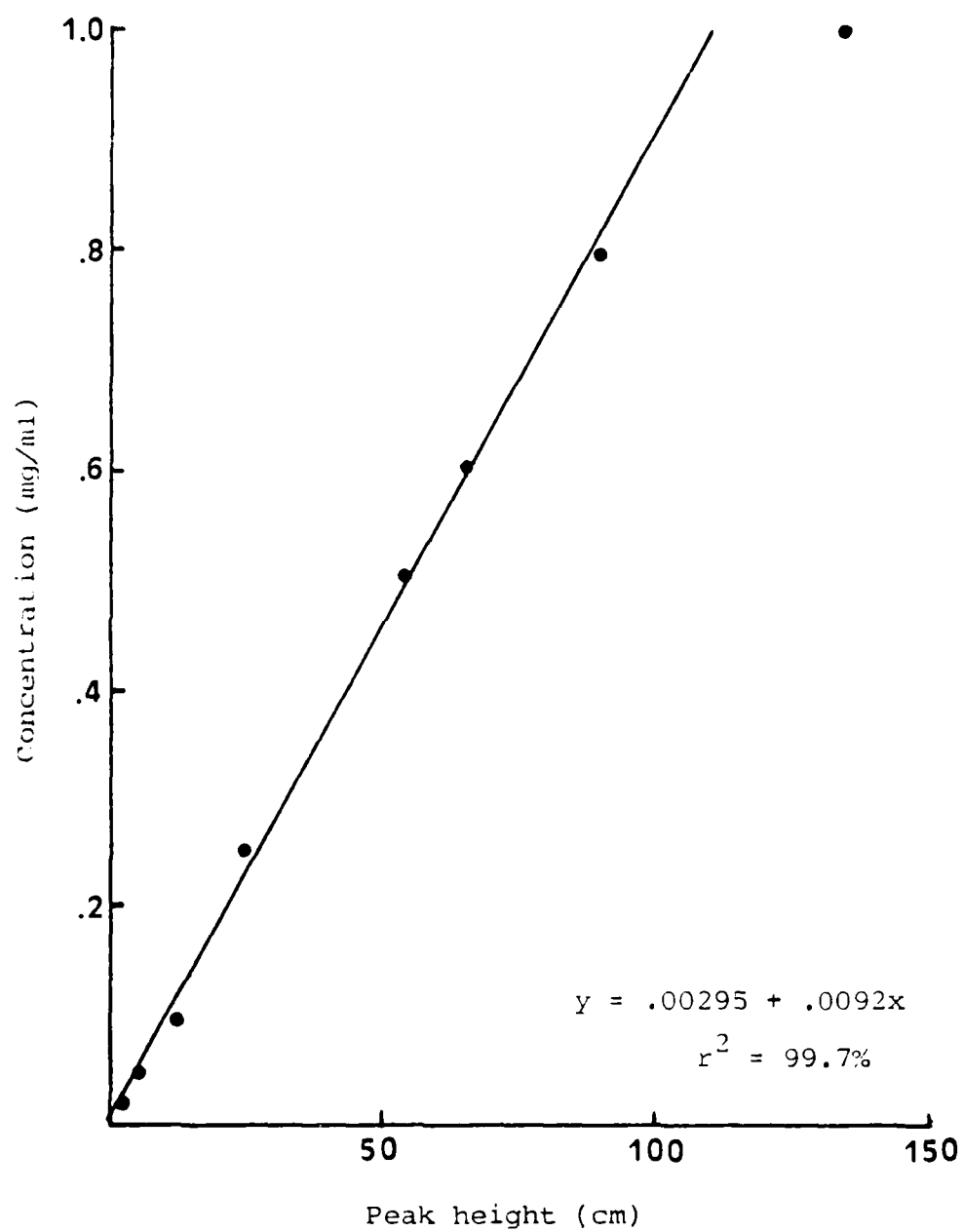(△) 2 gm/10ml frozen; (▲) 1 gm/10ml frozen; using
UV analysis.

Fresh solution   Frozen for 3 months   Refrigerated for 2 months
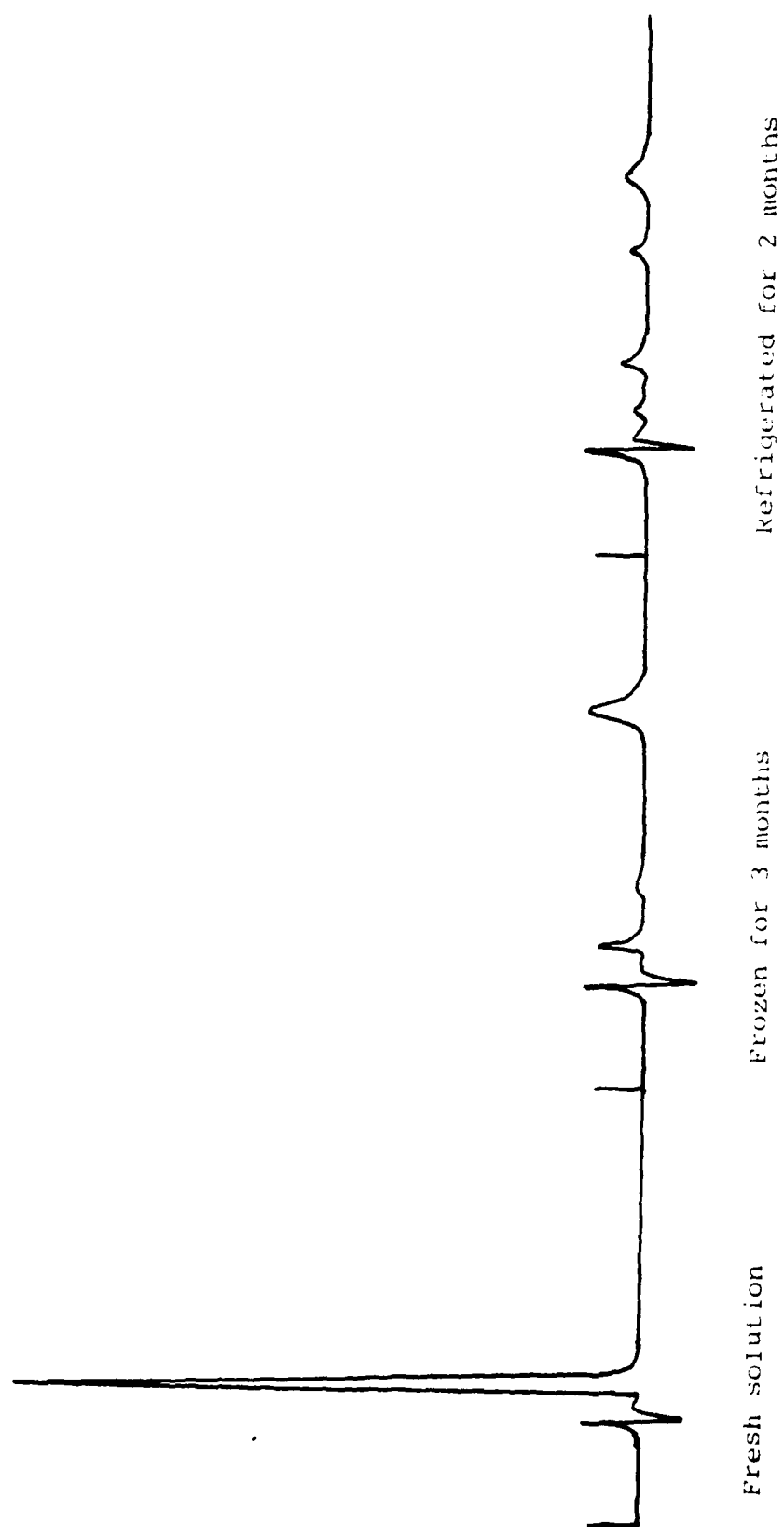
Figure IX A typical HPLC analysis of ampicillin

$$y = .00905 + .00793x$$
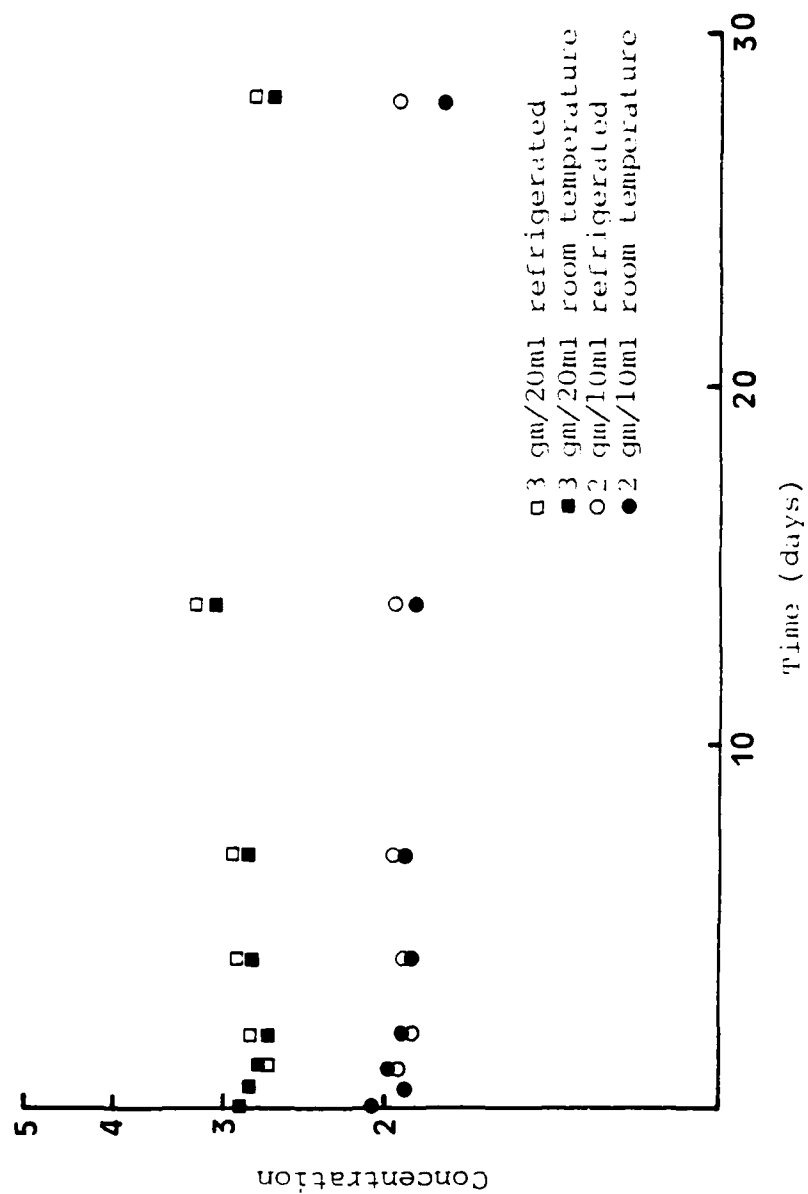$$r^2 = 99.7\%$$
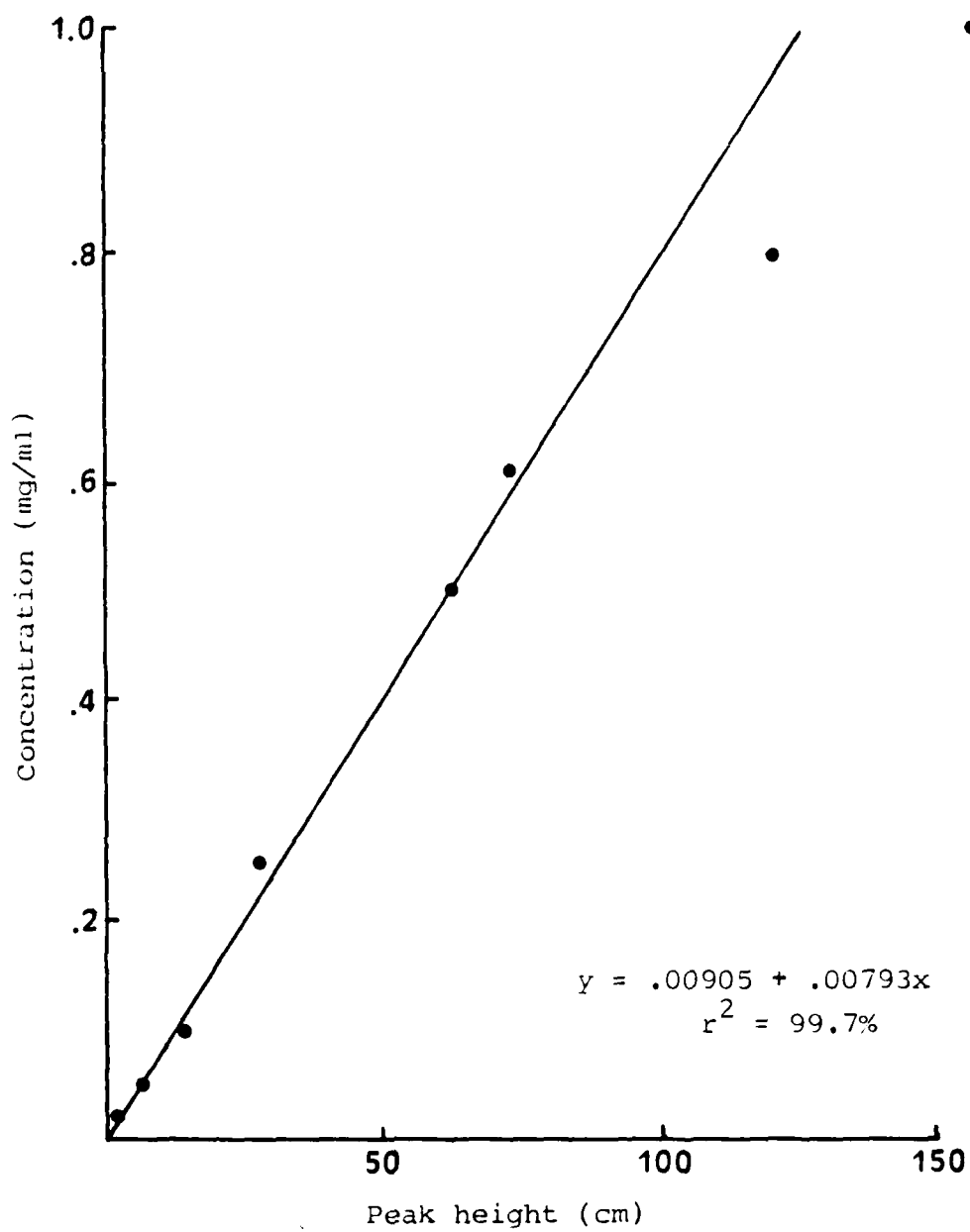
Figure X   Cefazolin Standard Curve
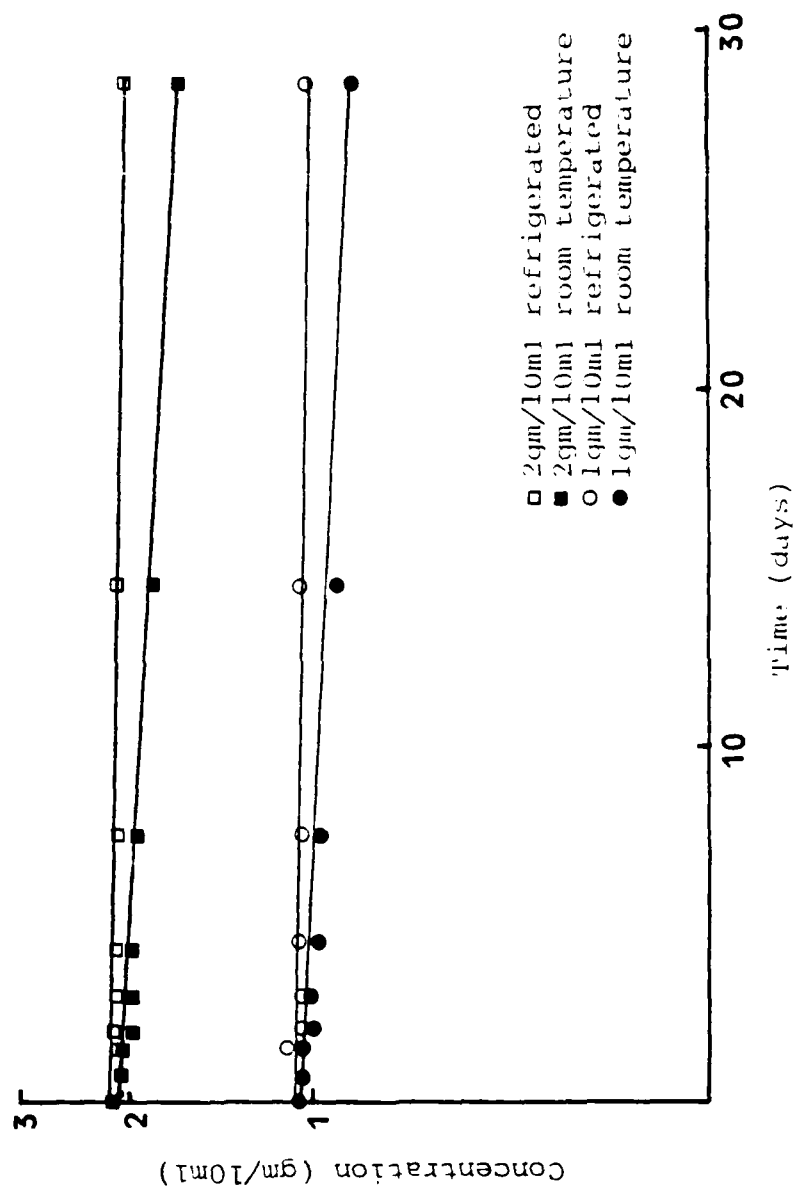
Figure XI   Concentration-time profile of cefazolin, using UV analysis.
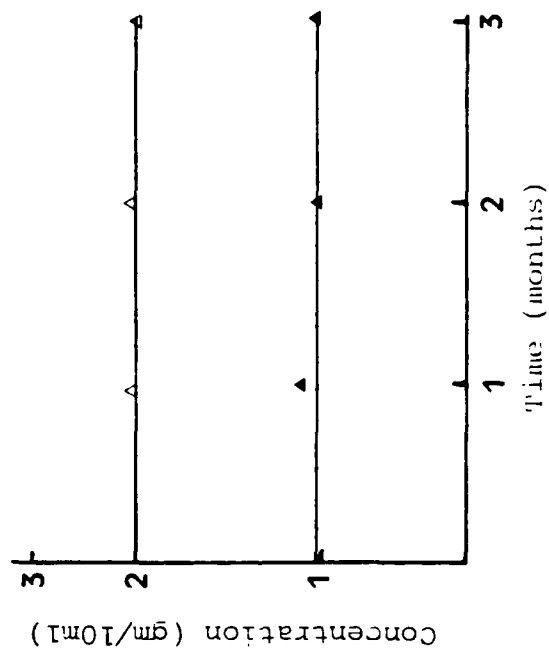
Figure XII   Concentration-time profile of cefazolin
(△) 2 gm/10ml frozen;   (▲) 1 gm/10ml frozen; using
UV analysis.

Fresh solution   Refrigerated 3 months   Frozen, then refrigerated
for 1 month
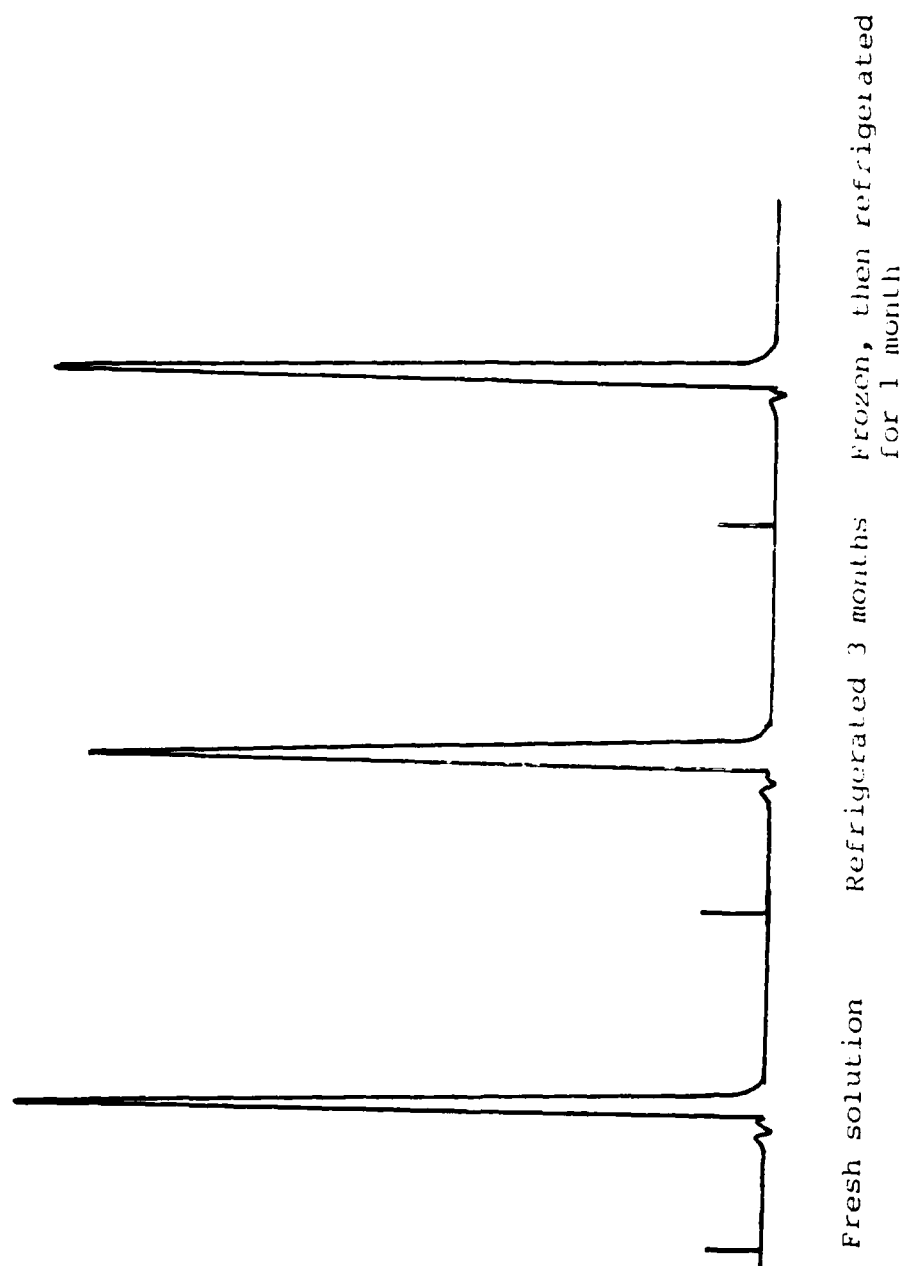
Figure XIII A typical HPLC analysis of cefazolin

$$y = .00305 + .00802x$$
$$r^2 = 99.9\%$$
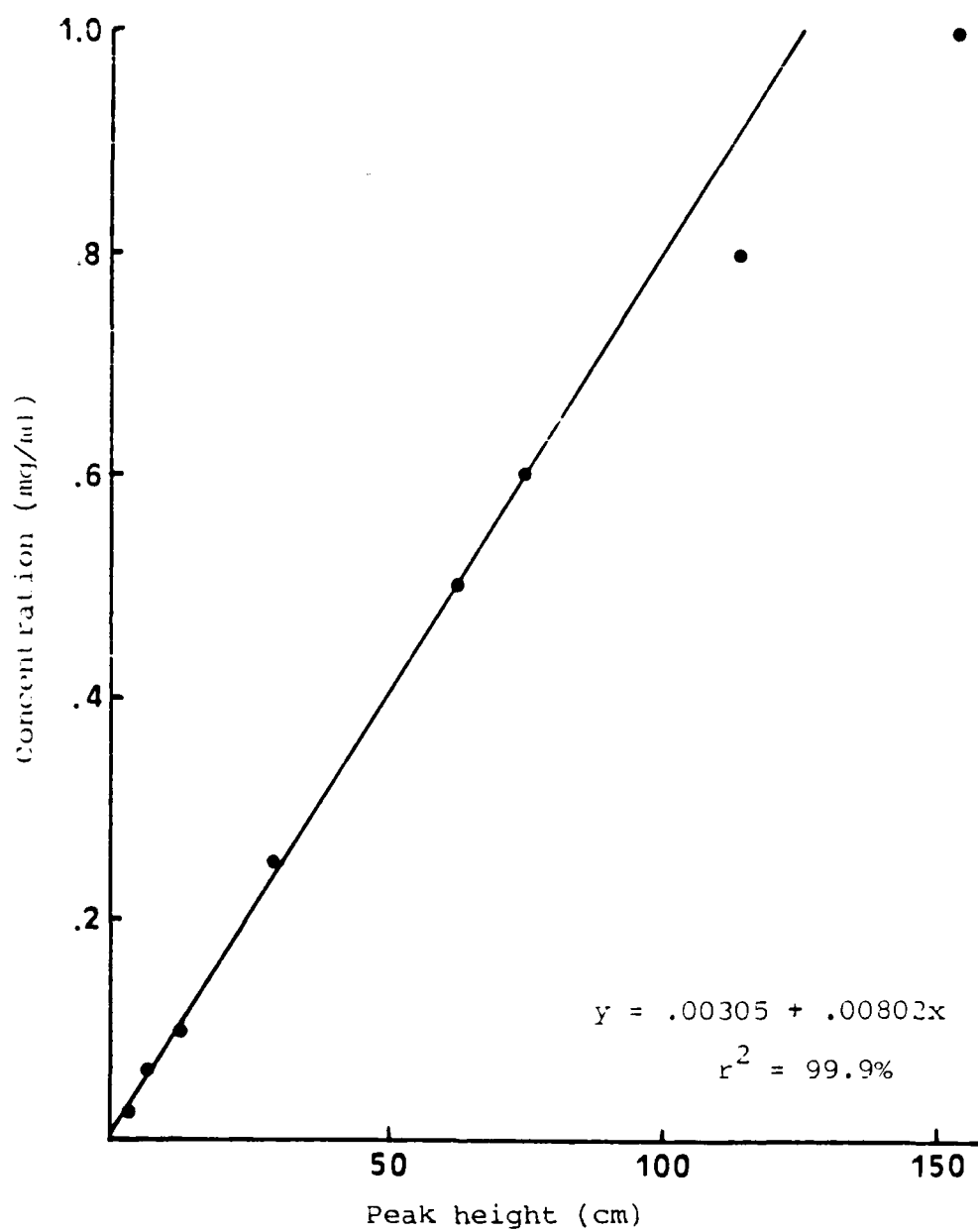
Figure XIV   Cefoxitin Standard Curve

Figure XV Concentration-time profile of cefoxitin, using UV analysis.
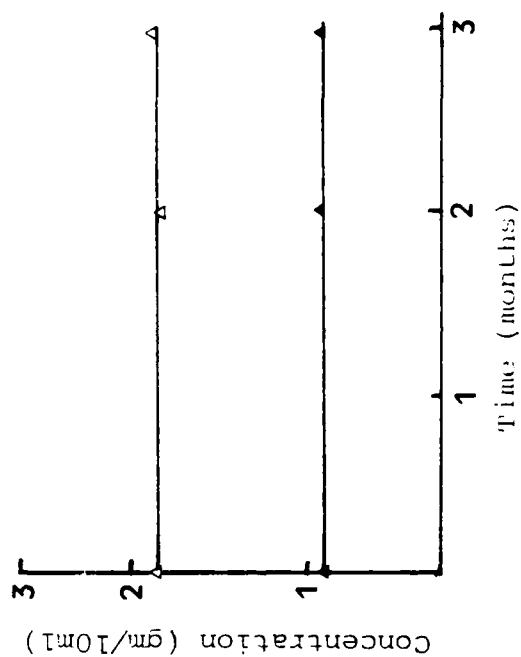
Figure XVI Concentration-time profile of cefoxitin
(Δ) 2 gm/10ml frozen; (▲) 1 gm/10ml frozen; using
UV analysis.

Figure XVII   Piperacillin Standard Curve

Figure XVIII Concentration-time profile of piperacillin, UV analysis.
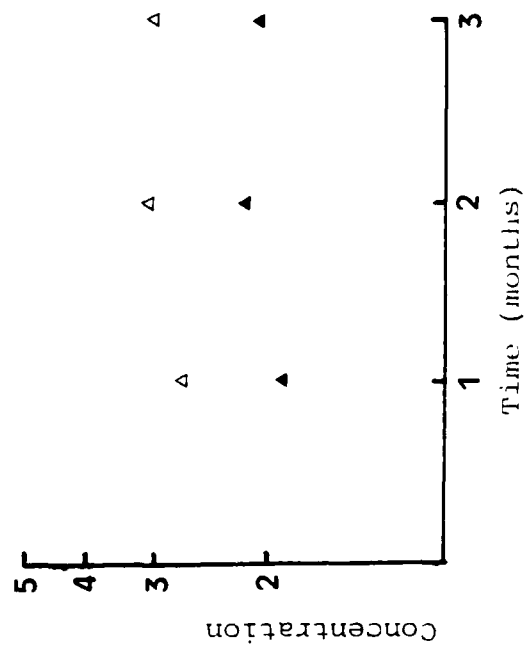
Figure XIX Concentration-time profile of piperacillin
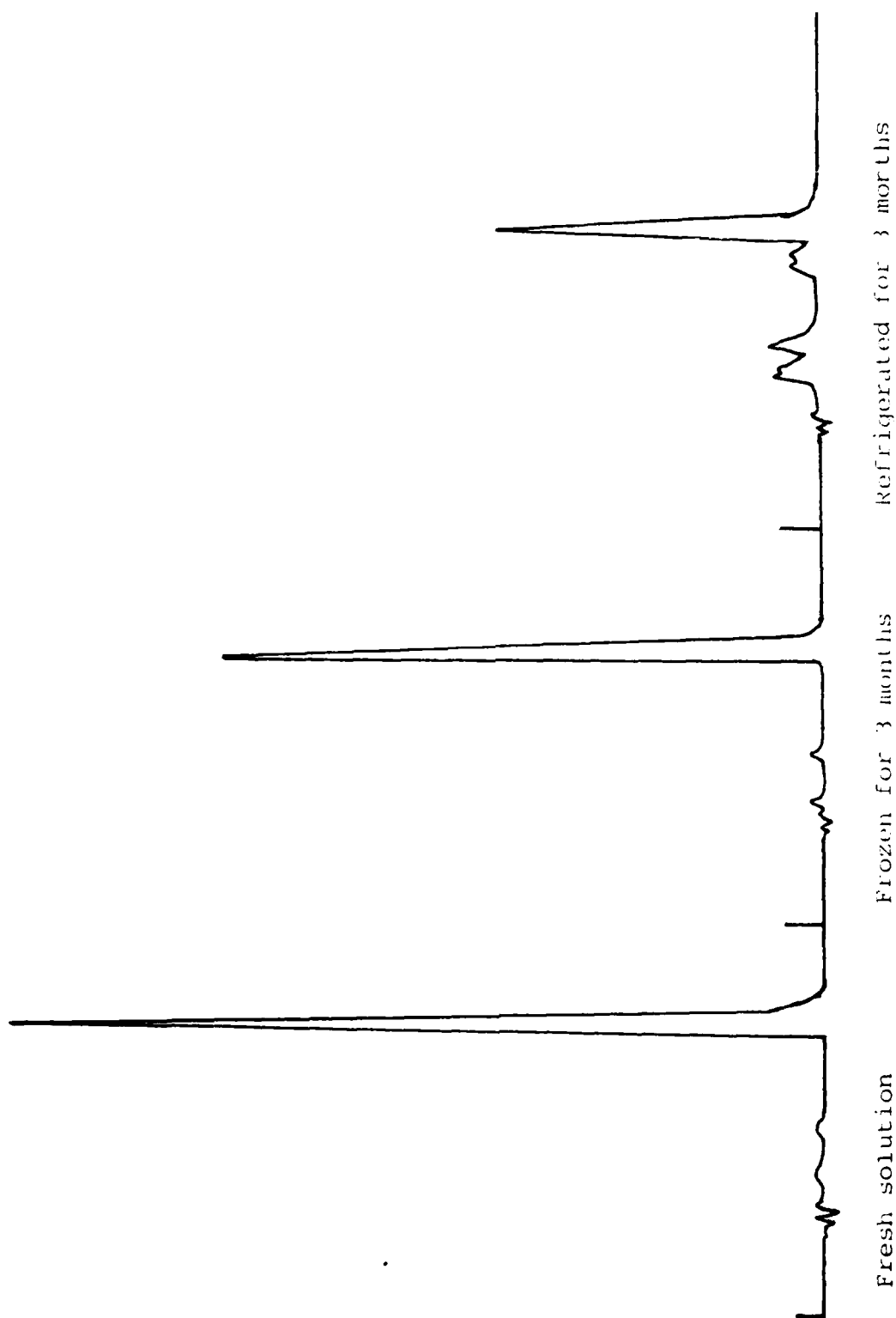(△) 3 gm/20ml frozen; (▲) 2 gm/10ml frozen; UV analysis.

Fresh solution   Frozen for 3 months   Refrigerated for 3 months

Figure XX   A typical HPLC analysis of piperacillin
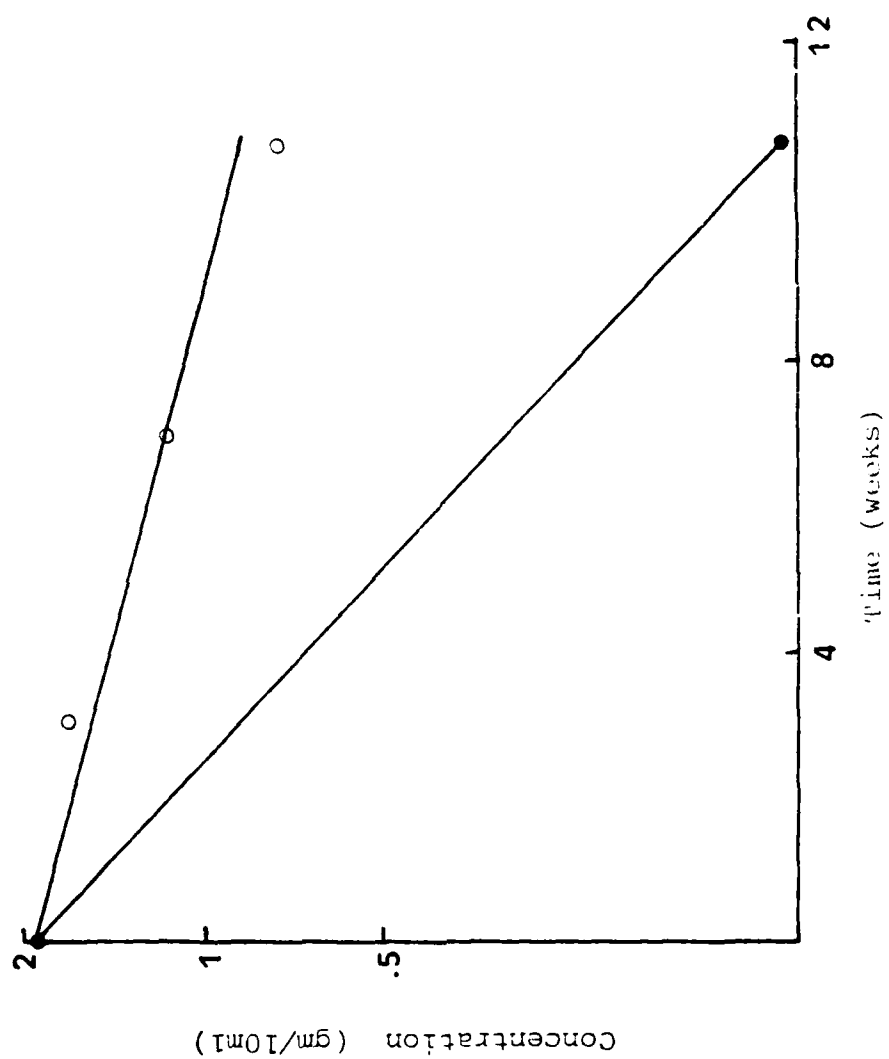
Figure XXI   Concentration-time profile of piperacillin
using HPLC.   (o) refrigerated;   (●) room temperature.
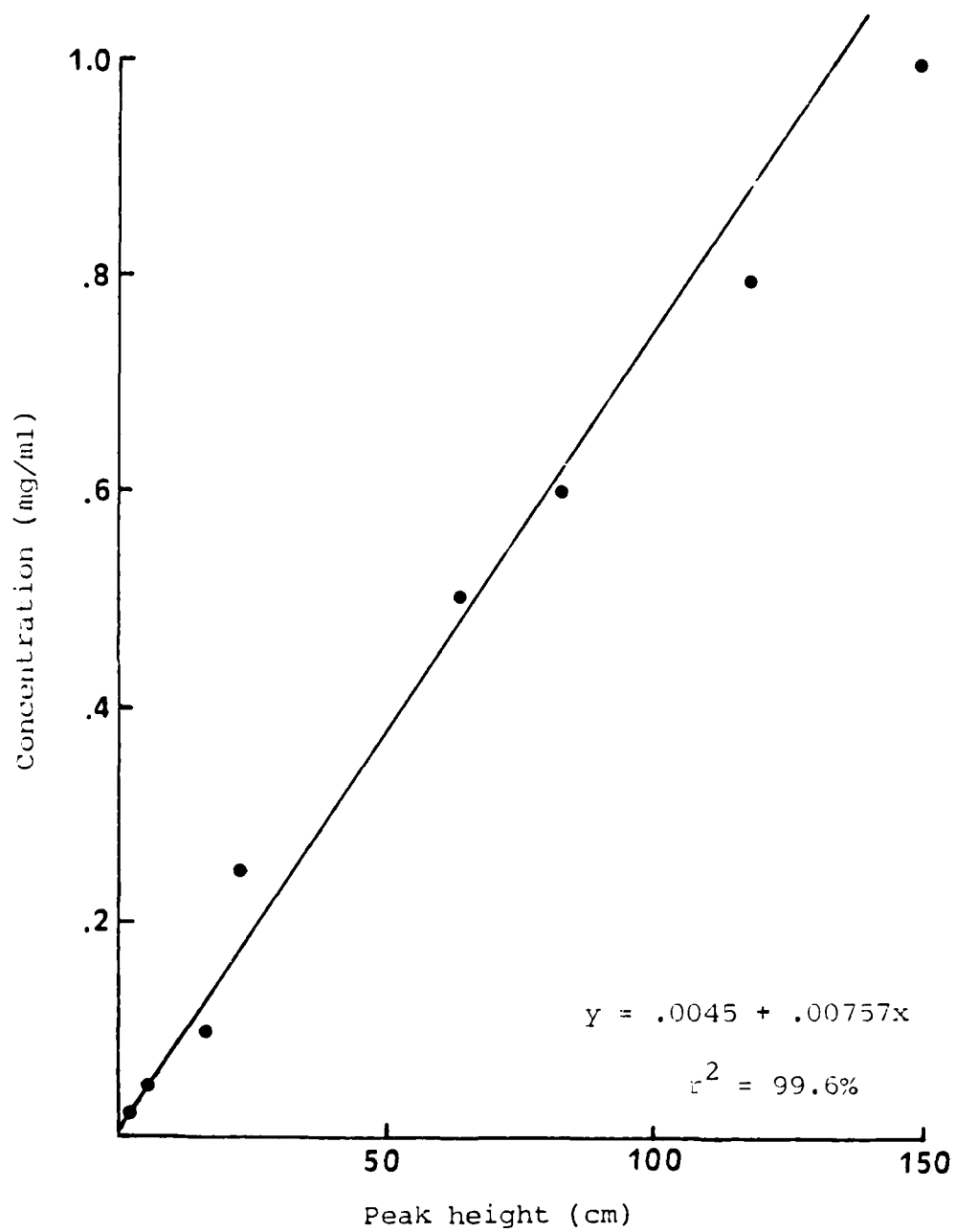
$$y = .0045 + .00757x$$

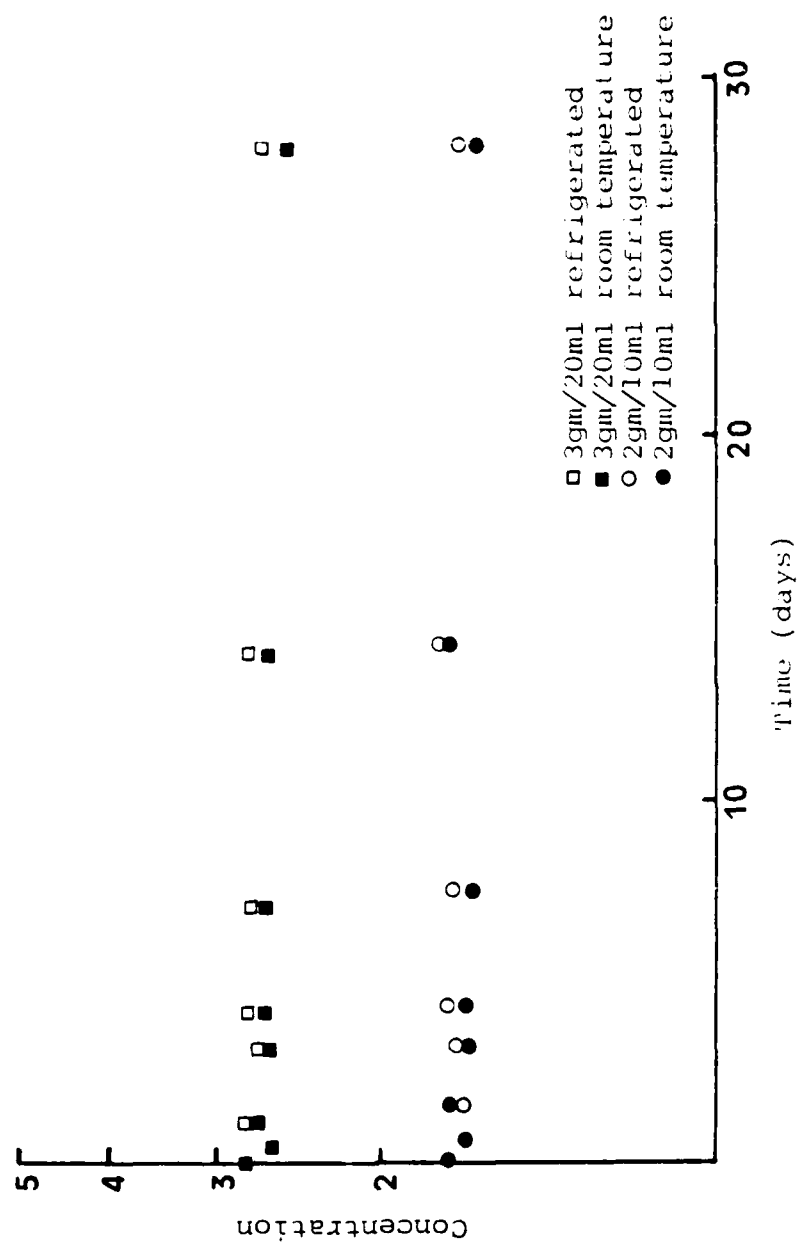$$r^2 = 99.6\%$$

Figure XXII Ticarcillin Standard Curve

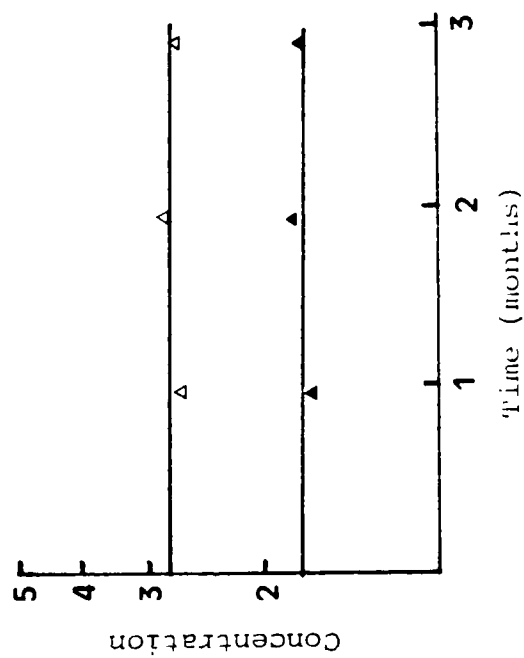Figure XXIII Concentration-time profile of ticarcillin, UV analysis.

Figure XXIV Concentration-time profile of ticarcillin,
(△) 3 gm/20ml frozen; (▲) 2 gm/10ml frozen; using
UV spectrophotometry.

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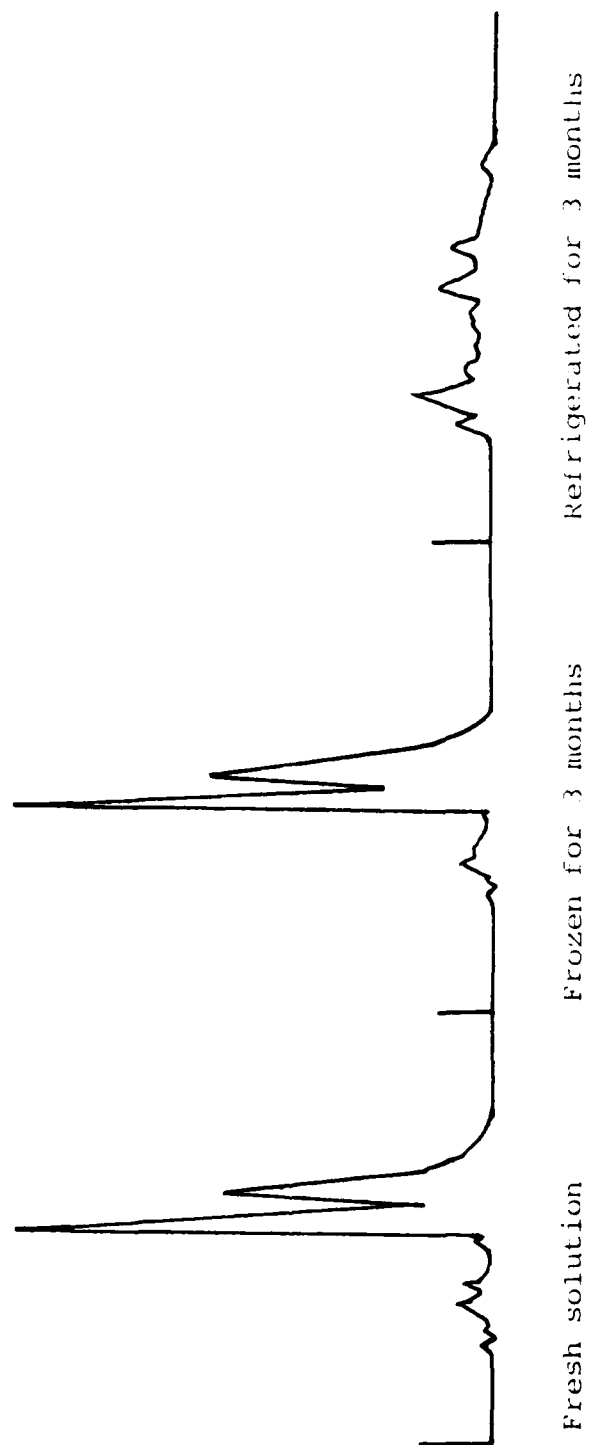Fresh solution   Frozen for 3 months   Refrigerated for 3 months

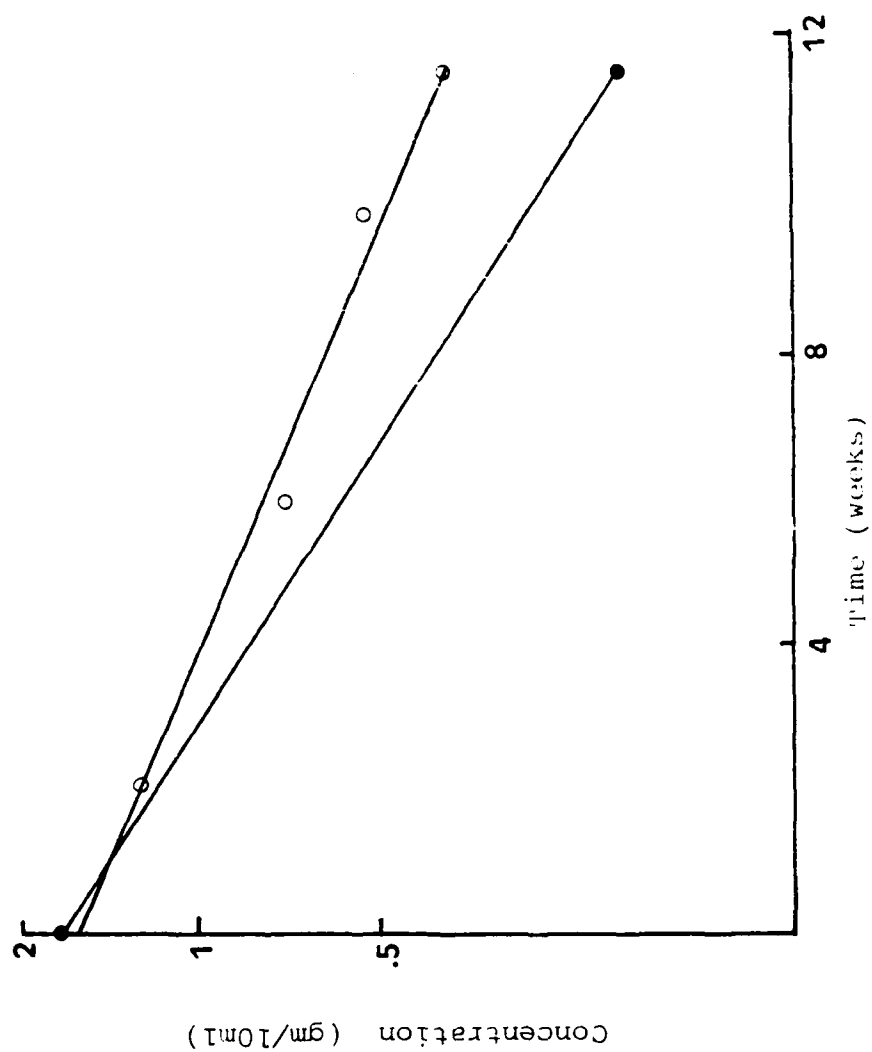Figure XXV   A typical HPLC anlaysis of ticarcillin.

Figure XXVI   Concentration-time profile of ticarcillin
using HPLC.   (O) refrigerated;   (●) room temperature.

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